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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	04/16/2024	11959109	Q289014	6959

23373 7590 03/27/2024
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at <https://patentcenter.uspto.gov/>. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at <https://certifiedcopycenter.uspto.gov/index.html>. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (<https://patentcenter.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional inventors):

Hikaru MIZUMURA, Tokyo, JAPAN;
Toshio Oda, Tokyo, JAPAN;
Shun-Ichiro Kawabata, Fukuoka, JAPAN;

APPLICANT(s) (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional applicants):

Seikagaku Corporation, Tokyo, JAPAN;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit [SelectUSA.gov](https://www.selectusa.gov).

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

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By fax, send to: (571) 273-2885

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(Note: Use Block 1 for any change of address)

23373

SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, NW, SUITE 9000
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Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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(Typed or printed name)

(Signature)

(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	Undiscounted	\$1200	\$0	\$1200	\$0	05/28/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
STEADMAN, DAVID J.	1656	435-023000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/ALA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

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1. Sughrue Mion, PLLC

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(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

SEIKAGAKU CORPORATION

Tokyo, Japan

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment (Please first reapply any previously paid fee shown above):

- ☐ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed Check ☐ Non-electronic payment by credit card (Attach form PTO-2038)
☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 19-4880

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29.

☐ Applicant asserting small entity status. See 37 CFR 1.27.

☐ Applicant changing to regular undiscounted fee status.

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NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /markboland/

Date March 6, 2024

Typed or Printed Name Mark Boland

Registration No. 32,197



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PATENT AND TRADEMARK OFFICE

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Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
03/06/2024 05:20:04 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6959	FILED BY	Kathleen Rowe
PATENT CENTER #	64578810	FILING DATE	07/03/2023
CUSTOMER #	23373	FIRST NAMED INVENTOR	Hikaru MIZUMURA
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	John Bird

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Q289014IssueFeeTransmittal.pdf	1	Issue Fee Payment (PTO-85B)	149 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Q289014IssueFeeTransmittal.pdf	5B5BFCBE7E4B0A93D150035C1030D19C7E6D12D718E38A71752EEDFB EF307DBF38C4F6CF0BC77A95FA35B0557F323A1B22CF43497AE3AC53D681DAFF1047411D

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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(Typed or printed name)

(Signature)

(Date)



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18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

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STEADMAN, DAVID J.	1656	435-023000

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1. Sughrue Mion, PLLC

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Tokyo, Japan

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- ☐ Applicant asserting small entity status. See 37 CFR 1.27.
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NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /markboland/

Date March 6, 2024

Typed or Printed Name Mark Boland

Registration No. 32,197



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NOTICE OF ALLOWANCE AND FEE(S) DUE

23373 7590 02/28/2024
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

EXAMINER

STEADMAN, DAVID J

ART UNIT

PAPER NUMBER

1656

DATE MAILED: 02/28/2024

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

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nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$1200.00	\$0	05/28/2024

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

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23373 7590 02/28/2024 SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 9000 WASHINGTON, DC 20006			EXAMINER STEADMAN, DAVID J	
			ART UNIT	PAPER NUMBER
			1656	
DATE MAILED: 02/28/2024				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).

<https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf>

Routine uses of the information in this record may include disclosure to:

- 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
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- 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
- 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
- 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
- 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
- 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
- 9) the Office of Personnel Management (OPM) for personnel research purposes; and
- 10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/346,420	Applicant(s) MIZUMURA et al.	
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the application papers filed on January 26, 2024.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-10 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a) ☒ All b) ☐ Some* c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☒ Certified copies of the priority documents have been received in Application No. 14/650,767 .
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.	6. ☐ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.	7. ☐ Other _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.	

/David Steadman/ Primary Examiner, Art Unit 1656	
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EXAMINER COMMENT

Status of the Application

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after allowance or after an Office action under *Ex Parte Quayle*, 25 USPQ 74, 453 O.G. 213 (Comm'r Pat. 1935). Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on January 26, 2024 has been entered.

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1-10 as set forth in the examiner's amendment filed on December 20, 2023 are pending in the application.

Information Disclosure Statement

The information disclosure statement (IDS) filed on January 26, 2024 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the IDS has been considered by the examiner. The documents cited on the IDS filed on January 26, 2024, either alone or in combination with the other prior art of record, do not teach or suggest the invention of claims 1-10 of this application.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

CPC						
Symbol					Type	Version
C12N	/	9	/	6408	F	2013-01-01
G01N	/	33	/	579	I	2013-01-01
C12Y	/	304	/	21069	I	2013-01-01
G01N	/	33	/	56911	I	2013-01-01
C12Y	/	304	/	21084	I	2013-01-01
G01N	/	2333	/	96411	A	2013-01-01

CPC Combination Sets					
Symbol		Type	Set	Ranking	Version
	/				

NONE (Assistant Examiner)		Total Claims Allowed: 10	
/David Steadman/ Primary Examiner, Art Unit 1656 (Primary Examiner)		22 February 2024 (Date)	O.G. Print Claim(s) 1
			O.G. Print Figure NONE

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.	
	Examiner DAVID J STEADMAN	Art Unit 1656	

INTERNATIONAL CLASSIFICATION			
CLAIMED			
C12N	/	9	/ 64
C12Q	/	1	/ 37
G01N	/	33	/ 579
NON-CLAIMED			
	/		/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE (Assistant Examiner) (Date)		Total Claims Allowed: 10	
/David Steadman/ Primary Examiner, Art Unit 1656 (Part of Paper No.: 20240221)		22 February 2024 (Date)	
(Part of Paper No.: 20240221)		O.G. Print Claim(s) 1	O.G. Print Figure NONE

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47															
CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE	Total Claims Allowed:	
(Assistant Examiner)	(Date)	10
/David Steadman/ Primary Examiner, Art Unit 1656	22 February 2024	O.G. Print Claim(s)
(Primary Examiner)	(Date)	1
		O.G. Print Figure
		NONE

<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

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<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

Search Notes		
Search Notes	Date	Examiner
PATENT DATA PORTAL inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
SEARCH inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
Reviewed prosecution history for priority applications 14/650,767, 15/983,725, 16/399,575, 16/399,386, 17/554,128	09/12/2023	/DJS/
SEARCH, STN database keyword search, see attached search printouts for search strategy and databases searched	09/12/2023	/DJS/
GOOGLE keyword search, see attached search printouts for search strategy	09/12/2023	/DJS/
Reviewed Global Dossier for applications EP13862466, EP18190686, and EP19216609	09/12/2023	/DJS/
C12N9/6408, C12Y304/21084 with keyword search, see SEARCH printout	09/12/2023	/DJS/
PATENT DATA PORTAL inventor name, applicant, and assignee search, all inventors searched	12/07/2023	/DJS/
PE2E SEARCH inventor name, applicant, and assignee search, all inventors searched	12/07/2023	/DJS/
Updated PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	12/07/2023	/DJS/
Updated C12N9/6408, C12Y304/21084 with keyword search, see PE2E SEARCH printout	12/07/2023	/DJS/
PATENT DATA PORTAL inventor name, applicant, and assignee search, all inventors searched	02/22/2024	/DJS/
PE2E SEARCH inventor name, applicant, and assignee search, all inventors searched	02/22/2024	/DJS/
Updated PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	02/22/2024	/DJS/
Updated C12N9/6408, C12Y304/21084 with keyword search, see PE2E SEARCH printout	02/22/2024	/DJS/

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<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	12/07/2023	/DJS/
	PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	02/22/2024	/DJS/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656
	Examiner Name	DAVID J STEADMAN
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1.	Third Party Observation dated December 14, 2022 submitted in corresponding European Patent Application No. 19216609.8 forwarded by EPO on December 19, 2022	
	2.	Third Party Observation dated July 17, 2023 submitted in corresponding European Patent Application No. 19216609.8 forwarded by EPO on July 20, 2023	
	3.	Office Action dated November 14, 2023 issued in corresponding European Patent Application No. 19216609.8	

EXAMINER SIGNATURE			
Examiner Signature	/David Steadman/	Date Considered	02/22/2024

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 1 of 2]

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /DJS/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656
	Examiner Name	DAVID J STEADMAN
	Attorney Docket Number	Q289014

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

- ☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

- ☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).
- ☐ See attached certification statement.
- ☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
- ☒ The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.
- ☒ A certification statement is not submitted herewith.

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Hui C. Wauters/	Date (YYYY-MM-DD)	2024-01-26
Name/Print	Hui C. Wauters	Registration Number	57,426

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 2 of 2]

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /DJS/

Bibliographic Data

Application No: 18/346,420

Foreign Priority claimed: ☒ Yes ☐ No

35 USC 119 (a-d) conditions met: ☒ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged:

/David Steadman/

Examiner's Signature

Initials

Title:

NOVEL RECOMBINANT FACTOR C AND METHOD FOR
PRODUCING THE SAME, AND METHOD FOR MEASURING
ENDOTOXIN

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/03/2023	435	1656	
RULE			

APPLICANTS

Seikagaku Corporation, Tokyo, JAPAN

INVENTORS

Hikaru MIZUMURA,

Toshio Oda,

Shun-Ichiro Kawabata, Fukuoka, JAPAN

CONTINUING DATA

This application is a CON of 17554128 12/17/2021

17554128 is a CON of 16399386 04/30/2019 PAT 11236318

16399386 is a CON of 15983725 05/18/2018 PAT 10982202

15983725 is a CON of 14650767 06/09/2015 PAT 10144923

14650767 is a 371 of PCT/JP2013/083082 12/10/2013

FOREIGN APPLICATIONS

JAPAN JP2012-269840 12/10/2012

IF REQUIRED, FOREIGN LICENSE GRANTED**

07/27/2023

STATE OR COUNTRY

ADDRESS

FILING FEE RECEIVED

\$6,160

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L5	7687	(horseshoe adj crab) OR limulus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 09:46 AM
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L14	6	L10 and (hek\$ OR (embryonic adj kidney))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:03 AM
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L20	18057	(mizumura-h\$ OR oda-	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13

		t\$ OR kawabata-s\$).in.	USOCR; FPRS; EPO; JPO; DERWENT)				09:23 AM
L21	20466	L19 or L20	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
L22	91	L21 and (horseshoe adj crab)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
L23	66	L21 and tridentatus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
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L25	108	L22 or L23 or L24	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:26 AM
L26	40	L25 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:26 AM
L27	23	("5,858,706" OR "5,712,144" OR "5,795,962" OR "6,077,946" OR "20090208995" OR "20030054432" OR "20040235080" OR "20140024062").pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 11:34 AM
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L29	4	"5,858,706".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 11:27 AM
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L31	2	"20140249082".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 04:45 PM
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L33	18751	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:07 PM
L34	21180	L32 or L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:07 PM
L35	19	L33 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM

L36	13	L35 and "factor C"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM
L37	2	L36 and (sialic OR sialyl\$)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM
L38	14664	"factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:53 PM
L39	218	L38 and (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:53 PM
L40	7	L38 same (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L41	218	L39 and (cho OR "chinese hamster ovary")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L42	805	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L43	2	L42 and (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L44	5676	(cho OR "chinese hamster ovary") same (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L45	211	L44 and L38	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L47	7	L45 and horseshoe	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:56 PM
L48	20	horseshoe and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:45 AM
L49	218	"factor c" and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:47 AM
L50	211	L49 not L48	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:47 AM
L51	0	L50 and horseshoe	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:48 AM
L52	2	"factor c".clm. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:48 AM
L53	6	"factor c".ti. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM

			JPO; DERWENT)				
L54	7	"factor c".ab. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM
L55	1	L54 not L53	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM
L56	2	"9133495".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:36 PM
L57	19132	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L58	6	L57 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L59	2485	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L60	3	L59 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L61	7	L58 or L60	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L62	19414	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L63	6	L62 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L64	2493	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L65	3	L64 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L66	7	L63 or L65	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L67	19635	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L68	6	L67 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L80	3	("20080131929" "20100086967" "20130295613").PN.	(US-PGPUB; USPAT)	OR	OFF	OFF	2018/09/07 10:58 AM
L81	19653	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM

			JPO; DERWENT)				
L82	6	L81 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM
L94	6	"10144923".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 09:23 AM
L96	6	(US-20080131929-\$ US-20100086967-\$ US-20130295613-\$).DID.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 10:53 AM
L97	20399	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L98	40	L97 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L99	23093	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:15 AM
L100	95	L99 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L101	54	L100 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L102	24	L101 and ("chinese hamster" OR CHO OR human OR HEK)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L103	627	tridentatus OR rotundicauda	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L104	389	L103 and endotoxin	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L105	263	L104 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L106	224	L103 same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L107	216	L105 and L106	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L108	140	L107 and "37"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L109	1050017	"2 hours"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L110	114	L108 and L109	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM

			USOCR; FPRS; EPO; JPO; DERWENT)				02:42 PM
L113	349173	cascade	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:44 PM
L114	169	L107 and L113	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:45 PM
L115	468	limulus adj test	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:30 PM
L116	1	L115 same cation	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:31 PM
L117	3	"6,645,724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 12:19 PM
L118	13	("20030054432" "20040 235080" "20080131929 " "20090208995" "2010 0086967" "2013029561 3" "20140024062" "201 40249082" "5712144" "5 716834" "5795962" "5 858706" "6077946").PN .	(US-PGPUB; USPAT)	OR	OFF	OFF	2020/04/22 02:32 PM
L119	23218	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L120	85	L119 AND (tridentatus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L121	54	L120 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:38 PM
L122	33	L121 and endotoxin.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:39 PM
L123	23351	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L124	95	L123 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L125	55	L124 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L126	10	L125 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L127	8	L125 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2020/06/24 03:12 PM

			JPO; DERWENT)				
L128	10	L126 or L127	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:12 PM
L129	23458	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L130	98	L129 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L131	57	L130 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L132	11	L131 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L133	9	L131 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L134	11	L132 or L133	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L135	753	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:52 AM
L136	44	L135 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L137	829430	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L138	104016	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L139	14	L136 and (L137 OR L138)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L140	4	"6645724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L141	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L142	46	L141 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L143	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L144	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM

			JPO; DERWENT)				
L145	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L146	46	L145 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L147	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L148	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L149	15	L146 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L150	64	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L151	24	L150 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L152	886720	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L153	112690	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L154	69	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L155	19	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L156	812	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L157	50	L156 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L158	16	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L159	40	L156 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:30 AM
L160	13	(L152 OR L153) same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:31 AM
L161	204	(L152 OR L153) same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2021/10/18 08:40 AM

			JPO; DERWENT)				
L162	13	L160 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:41 AM
L163	4875	(hek OR hek293 OR (human adj embryo\$)).clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L164	8	L157 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L165	14	L154 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L166	6	L165 not L164	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L167	8	hek293 same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:50 AM
L168	24311	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L169	103	L168 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L170	63	L169 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L171	15	L170 and (L152 or L153 OR hek293)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:52 AM
L172	2183	ding-J\$.in.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L173	22336	"factor c"	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L174	26	172 AND 173	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L175	722713	secret\$	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L176	13	174 AND 175	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L177	563	tachypleus	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L178	172826	ding-J\$.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2023/03/13 03:56 PM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L179	81001	"factor c"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:56 PM
L180	117	178 AND 179	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L181	2156	tachypleus	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM

L182	17	180 AND 181	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L183	3284055	secret\$	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L184	13	182 AND 183	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L185	93965	"factor b"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2023/03/14 09:54 AM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L188	438	185 AND 177	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:55 AM
L189	3617370	"chinese hamster" OR CHO????	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM
L190	104	188 AND 189	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM

L191	21	recombinant?? adj5 185	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 10:04 AM
L192	12	("0000001" OR "10144923" OR "20020068325" OR "20030054432" OR "20040235080" OR "20080131929" OR "20090208995" OR "20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5795962").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/14 05:22 PM
L193	21	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 05:59 PM
L194	115	L156 AND (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L195	10351	limulus OR tachypleus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L196	25	194 AND 195	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L197	9	196 NOT 193	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L198	2271	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:49 AM
L199	25141	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L200	27	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L201	379035	(human ADJ embryo\$) OR hek???	(US-PGPUB; USPAT; USOCR; FIT (AP, AT,	OR	ON	ON	2023/03/15 05:51 AM

			AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L202	3817922	189 OR 201	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:51 AM
L203	37	200 AND 202	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:52 AM
L204	28	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/11 03:26 PM
L205	19	("10144923" OR "10982202" OR "11236318" OR "20020068325" OR "20030054432" OR "20040235080" OR "20080131929" OR	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/09/11 04:10 PM

		"20090208995" OR "20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5712144" OR "5716834" OR "5795962" OR "5840510" OR "5858706" OR "6077946" OR "6645724").pn.					
L206	1	("6733997").pn.	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/09/11 04:10 PM
L207	22	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:10 AM
L208	24	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM
L209	29	207 OR 208	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM
L210	26980	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as,aanm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:33 AM
L211	29	198 AND 210	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:34 AM
L212	22825	((human ADJ embryo\$) OR hek???.clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:34 AM
L213	368450	("chinese hamster" OR CHO????).clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL,	OR	ON	ON	2023/12/07 05:35 AM

			NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L214	18	211 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:35 AM
L215	10356	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2023/12/07 05:40 AM
L216	177	215 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:41 AM
L217	174705	horseshoe	(US-PGPUB; USPAT;	OR	ON	ON	2023/12/07

			USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				05:41 AM
L218	25	216 AND 217	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:41 AM
L219	54	L145 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:47 AM
L220	34	L154 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:48 AM
L221	55	219 OR 220	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:48 AM
L222	23	221 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:48 AM

L223	29	198 AND 210	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2024/02/22 05:45 AM
L225	2	223 AND secret\$.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2024/02/22 05:47 AM
L226	24	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2024/02/22 06:28 AM
L227	26	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2024/02/22 06:28 AM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N69	2075	(dg44 OR dg-44 OR chodg44 OR cho-dg44)	(USPAT)	OR	OFF	OFF	2018/08/14 09:27 AM
N70	5851	"factor c"	(USPAT)	OR	OFF	OFF	2018/08/14 09:27 AM
N71	92	N69 and N70	(USPAT)	OR	OFF	OFF	2018/08/14 09:27 AM
N72	17574	horseshoe OR crab	(USPAT)	OR	OFF	OFF	2018/08/14 09:28 AM
N73	2	N71 and N72	(USPAT)	OR	OFF	OFF	2018/08/14 09:28 AM
N74	40	(dg44 OR dg-44 OR chodg44 OR cho-dg44).clm.	(USPAT)	OR	OFF	OFF	2018/08/14 09:30 AM
N75	380	"factor c".clm.	(USPAT)	OR	OFF	OFF	2018/08/14 09:30 AM
N76	0	N74 and N75	(USPAT)	OR	OFF	OFF	2018/08/14 09:30 AM
N77	2254	(horseshoe OR crab).clm.	(USPAT)	OR	OFF	OFF	2018/08/14 09:31 AM
N78	0	N74 and N77	(USPAT)	OR	OFF	OFF	2018/08/14 09:31 AM
N79	0	(dg44 OR dg-44 OR chodg44 OR cho-dg44) same "factor c"	(USPAT)	OR	OFF	OFF	2018/08/14 09:31 AM
N83	2086	(dg44 OR dg-44 OR chodg44 OR cho-dg44)	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N84	5873	"factor c"	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N85	92	N83 and N84	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N86	17635	horseshoe OR crab	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N87	2	N85 and N86	(USPAT)	OR	OFF	OFF	2018/09/07

							11:44 AM
N88	41	(dg44 OR dg-44 OR chodg44 OR cho-dg44).clm.	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N89	383	"factor c".clm.	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N90	0	N88 and N89	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N91	2260	(horseshoe OR crab).clm.	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N92	0	N88 and N91	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N93	0	(dg44 OR dg-44 OR chodg44 OR cho-dg44) same "factor c"	(USPAT)	OR	OFF	OFF	2018/09/07 11:44 AM
N173	45	(tridentatus OR polyphemus).clm.	(USPAT)	OR	OFF	OFF	2021/10/18 08:47 AM
N174	455	"factor c".clm.	(USPAT)	OR	OFF	OFF	2021/10/18 08:47 AM
N175	9209	(cho OR "chinese hamster").clm.	(USPAT)	OR	OFF	OFF	2021/10/18 08:47 AM
N176	43441	(hek OR hek293 OR (human adj embryo\$))	(USPAT)	OR	OFF	OFF	2021/10/18 08:54 AM
N177	51060	N175 or N176	(USPAT)	OR	OFF	OFF	2021/10/18 08:55 AM
N178	2	N173 and N174 and N177	(USPAT)	OR	OFF	OFF	2021/10/18 08:55 AM
N179	23	N174 and N177	(USPAT)	OR	OFF	OFF	2021/10/18 08:56 AM
N180	21	N179 not N178	(USPAT)	OR	OFF	OFF	2021/10/18 08:56 AM
N181	1502	(hek OR hek293 OR (human adj embryo\$)).clm.	(USPAT)	OR	OFF	OFF	2021/10/18 08:58 AM
N182	10237	N175 or N181	(USPAT)	OR	OFF	OFF	2021/10/18 08:58 AM
N183	4	N174 and N182	(USPAT)	OR	OFF	OFF	2021/10/18 08:58 AM
N184	2	N173 and N182	(USPAT)	OR	OFF	OFF	2021/10/18 08:59 AM
N185	4	N183 or N184	(USPAT)	OR	OFF	OFF	2021/10/18 08:59 AM
N186	16	rotundicauda.clm.	(USPAT)	OR	OFF	OFF	2021/10/18 10:40 AM
N187	9209	(cho OR "chinese hamster").clm.	(USPAT)	OR	OFF	OFF	2021/10/18 10:41 AM
N188	43441	(hek OR hek293 OR (human adj embryo\$))	(USPAT)	OR	OFF	OFF	2021/10/18 10:41 AM
N189	51060	N187 or N188	(USPAT)	OR	OFF	OFF	2021/10/18

							10:41 AM
N190	2	N186 and N189	(USPAT)	OR	OFF	OFF	2021/10/18 10:41 AM
N191	247408	(cho OR "chinese hamster")	(USPAT)	OR	OFF	OFF	2021/10/18 10:42 AM
N192	247408	N187 or N191	(USPAT)	OR	OFF	OFF	2021/10/18 10:42 AM
N193	2	N186 and N192	(USPAT)	OR	OFF	OFF	2021/10/18 10:42 AM
N194	1354	"factor c".clm.	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/12/07 05:37 AM
N195	53992	("chinese hamster" OR CHO????).clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:38 AM
N196	4438	((human ADJ embryo\$) OR hek???).clm.	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:38 AM
N198	57914	195 OR 196	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:38 AM
N199	44	194 AND 198	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:39 AM
N200	30038	horseshoe	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:44 AM
N201	10	199 AND 200	(US-PGPUB; USPAT)	OR	ON	ON	2023/12/07 05:44 AM
N202	10	199 AND 200	(US-PGPUB; USPAT)	OR	ON	ON	2024/02/22 05:49 AM
N203	61889	secret\$.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2024/02/22 05:58 AM
N204	69	203 AND 194	(US-PGPUB; USPAT)	OR	ON	ON	2024/02/22 05:59 AM
N205	12	198 AND 204	(US-PGPUB; USPAT)	OR	ON	ON	2024/02/22 06:00 AM



UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
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GRANT LETTER

APPLICATION #	DECISION DATE	DECISION ON PETITION #	ATTORNEY DOCKET NUMBER
18/346,420	01/26/2024	37 CFR 1.313(c)(2)	Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

In re Application of Hikaru MIZUMURA

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed January 26, 2024 to withdraw the above-identified application from issue after payment of the issue fee.

The request is **GRANTED**.

The above-identified application is withdrawn from issue for consideration of a submission under 37 CFR 1.114 (request for continued examination). See 37 CFR 1.313(c)(2).

Petitioner is advised that the issue fee paid in this application cannot be refunded. If, however, this application is again allowed, petitioner may request that it be applied towards the issue fee required by the new Notice of Allowance.

The application is being referred to Technology Center AU for processing of the request for continuing examination under 37 CFR 1.114 .

Telephone inquired concerning this decision should be directed to the Patent Electronic Business Center (EBC) at 866-217-9197.

Office of Petitions

REQUEST FOR CONTINUED EXAMINATION (RCE) TRANSMITTAL							
Application Number	18/346,420	Filing Date	July 3, 2023	Confirmation Number	6959	Docket Number	Q289014
First Named Inventor	Hikaru MIZUMURA	Examiner Name	DAVID J STEADMAN			Art Unit	1656
Title	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN						
This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.							
SUBMISSION REQUIRED UNDER 37 CFR 1.114							
<small>Note: If the RCE is proper, any previously filed unentered amendments and amendments attached with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).</small>							
☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____ ☐ Other _____							
☐ Do NOT consider the amendment(s)/reply under 37 CFR 1.116 previously filed on _____							
☒ Attached ☐ Amendment/Reply ☒ Information Disclosure Statement (IDS) ☐ Affidavit(s)/Declaration(s) ☒ Other <u>Petition to Withdraw from Issuance</u>							
MISCELLANEOUS							
☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)							
☐ Other _____							
FEES							
The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed. ☒ Payment by credit card. ☒ The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.							
CORRESPONDENCE ADDRESS							
<i>Direct all correspondence to the address for SUGHRUE MION, PLLC filed under the Customer Number listed below:</i> <small>WASHINGTON OFFICE</small> <div style="font-size: 1.5em; font-weight: bold;">23373</div> <small>CUSTOMER NUMBER</small>							
SIGNATURE OF REGISTERED U.S. PATENT PRACTITIONER							
Signature <u>/Hui C. Wauters/</u>				Date <u>January 26, 2024</u>			
Name <u>Hui C. Wauters</u>				Registration No. <u>57,426</u>			



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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
01/26/2024 12:17:02 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	11891639
CONFIRMATION #	6959	FILED BY	Hui Wauters
PATENT CENTER #	64099679	FILING DATE	07/03/2023
CUSTOMER #	23373	FIRST NAMED INVENTOR	Hikaru MIZUMURA
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	-

Documents

TOTAL DOCUMENTS: 7

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
petition-request.pdf	2	ePetition Request Form	29 KB
Q289014RCEasfiled.pdf	1	Request for Continued Examination (RCE)	45 KB
Warning: This is not a USPTO supplied RCE fillable form. Data in the form cannot be automatically loaded to other USPTO systems.			
Q289014IDSasfiled.pdf	4	-	60 KB
Q289014IDSasfiled-TRAN.LET.pdf	(1-2) 2	Transmittal Letter	28 KB
Q289014IDSasfiled-IDS.pdf	(3-4) 2	Information Disclosure Statement (IDS) Form (SB08)	41 KB
Q289014NPLREFThirdParty	12	Non Patent Literature	2253 KB

Observationdated12142022.
pdf

Q289014NPLREFEPOfficeA
ctiondated11142023.pdf

7

Non Patent Literature

869 KB

Q289014NPLREFThirdParty
Observationdated07172023.
pdf

8

Non Patent Literature

1532 KB

Digest

DOCUMENT

MESSAGE DIGEST(SHA-512)

petition-request.pdf

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Q289014RCEasfiled.pdf

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Q289014IDSasfiled.pdf

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Q289014NPLREFThirdPartyOb
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Q289014NPLREFThirdPartyOb
servatiodated07172023.pdf

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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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PATENT AND TRADEMARK OFFICE

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
01/26/2024 12:17:02 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	11891639
CONFIRMATION #	6959	FILED BY	Hui Wauters
PATENT CENTER #	64099679	AUTHORIZED BY	-
CUSTOMER #	23373	FILING DATE	07/03/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Hikaru MIZUMURA

Payment Information

PAYMENT METHOD DA / 194880	PAYMENT TRANSACTION ID E20241PC18215343	PAYMENT AUTHORIZED BY Hui Wauters
PRE-AUTHORIZED ACCOUNT 194880	PRE-AUTHORIZED CATEGORY 37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees); 37 CFR 1.19 (Document supply fees); 37 CFR 1.21 (Miscellaneous fees and charges)	

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1464	PETITIONS REQUIRING THE PETITION FEE SET FORTH IN 37 CFR 1.17(H) (GROUP III)	140.00	1	140.00
1801	REQUEST FOR CONTINUED EXAMINATION (RCE) - 1ST REQUEST (SEE 37 CFR 1.114)	1360.00	1	1360.00
TOTAL AMOUNT:				\$1,500.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized

by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q289014
Hikaru MIZUMURA, et al.	Allowed: December 20, 2023
Appln. No.: 18/346,420	Group Art Unit: 1656
Confirmation No.: 6959	Examiner: DAVID J STEADMAN
Filed: July 3, 2023	

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

**INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§ 1.97 and 1.98**

MAIL STOP ISSUE FEE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure under 37 C.F.R. § 1.56, Applicant hereby notifies the U.S. Patent and Trademark Office of the documents which are listed on the attached PTO/SB/08 (modified) form and/or listed herein.

One copy of each of the listed documents is submitted herewith.

The present Information Disclosure Statement is being filed: (1) No later than three months from the application's filing date; (2) Before the mailing date of the first Office Action on the merits (whichever is later); or (3) Before the mailing date of the first Office Action after filing a request for continued examination (RCE) under §1.114, and therefore, no Statement under 37 C.F.R. § 1.97(e) or fee under 37 C.F.R. § 1.17(p) is required.

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not

INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§ 1.97 and 1.98
Appln. No.: 18/346,420

Attorney Docket No.: Q289014

waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/Hui C. Wauters/

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: January 26, 2024

Hui C. Wauters
Registration No. 57,426

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656
	Examiner Name	DAVID J STEADMAN
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1.	Third Party Observation dated December 14, 2022 submitted in corresponding European Patent Application No. 19216609.8 forwarded by EPO on December 19, 2022	
	2.	Third Party Observation dated July 17, 2023 submitted in corresponding European Patent Application No. 19216609.8 forwarded by EPO on July 20, 2023	
	3.	Office Action dated November 14, 2023 issued in corresponding European Patent Application No. 19216609.8	

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 1 of 2]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656
	Examiner Name	DAVID J STEADMAN
	Attorney Docket Number	Q289014

CERTIFICATION STATEMENT			
Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):			
☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).			
OR			
☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).			
☐ See attached certification statement.			
☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.			
☒ The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.			
☒ A certification statement is not submitted herewith.			
WASHINGTON OFFICE 23373 CUSTOMER NUMBER			
SIGNATURE			
A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.			
Signature	/Hui C. Wauters/	Date (YYYY-MM-DD)	2024-01-26
Name/Print	Hui C. Wauters	Registration Number	57,426

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 2 of 2]



UNITED STATES
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PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c)(2) with Assigned Patent Number)

APPLICATION #
18346420

ATTORNEY DOCKET #
Q289014

FILING DATE
07/03/2023

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD
FOR MEASURING ENDOTOXIN

Application Information

CONFIRMATION # **6959**

FILING DATE **07/03/2023**

ART UNIT **1656**

FIRST NAMED INVENTOR **Hikaru MIZUMURA**

EXAMINER **DAVID STEADMAN**

An application may be withdrawn from issue for further action upon petition by the applicant. To request that the Office withdraw an application from issue, applicant must file a petition under this section including the fee set forth in § 1.17(h) and a showing of good and sufficient reasons why withdrawal of the application from issue is necessary.

APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).

A grantable petition requires the following items:

- (1) Petition fee; and
- (2) One of the following reasons: A grantable petition requires the following items: (a) Unpatentability of one or more claims, which must be accompanied by an unequivocal statement that one or more claims are unpatentable, an amendment to such claim or claims, and an explanation as to how the amendment causes such claim or claims to be patentable;
- (b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or
- (c) Express abandonment of the application. Such express abandonment may be in favor of a continuing application, but not a CPA under 37 CFR 1.53(d).

Reason for withdrawal

Consideration of a request for continued examination - RCE request, submission, and fee are attached

Applicant claims the following entity status

Regular Undiscounted

Signature

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

An attorney or agent registered to practice before the Patent and Trademark Office who has been given power of attorney in this application

Signature

Name

Registration #

/Hui C. Wauters/

Hui Wauters

57426



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	02/06/2024	11891639	Q289014	6959

23373 7590 01/17/2024
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at <https://patentcenter.uspto.gov/>. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at <https://certifiedcopycenter.uspto.gov/index.html>. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (<https://patentcenter.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional inventors):

Hikaru MIZUMURA, Tokyo, JAPAN;
Toshio Oda, Tokyo, JAPAN;
Shun-Ichiro Kawabata, Fukuoka, JAPAN;

APPLICANT(s) (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional applicants):

Seikagaku Corporation, Tokyo, JAPAN;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit [SelectUSA.gov](https://www.selectusa.gov).



UNITED STATES
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
12/21/2023 03:52:51 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Joji Pantoja

PATENT CENTER # 63712223

FILING DATE 07/03/2023

CUSTOMER # 23373

FIRST NAMED INVENTOR Hikaru MIZUMURA

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Abraham Rosner

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Q289014TransmittalIssueFee.pdf	1	Issue Fee Payment (PTO-85B)	149 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Q289014TransmittalIssueFee.pdf	A7D56BA347CE4FA4F221C18A8891637F3F1DEF6D9DEB59382 B89B4E44541F6B4707EFD9A0988C9B1B5F9B8B4DA4213A969 51E77B177593D95380BAED30C54B54

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
12/21/2023 03:52:51 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Joji Pantoja

PATENT CENTER # 63712223

AUTHORIZED BY Abraham Rosner

CUSTOMER # 23373

FILING DATE 07/03/2023

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Hikaru MIZUMURA

Payment Information

PAYMENT METHOD
CARD / 7940

PAYMENT TRANSACTION ID
E2023BKF53466057

PAYMENT AUTHORIZED BY
Joji Pantoja

PRE-AUTHORIZED ACCOUNT
194880

PRE-AUTHORIZED CATEGORY
37 CFR 1.20 (Post Issuance fees)

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1501	UTILITY ISSUE FEE	1200.00	1	1200.00
TOTAL AMOUNT:				\$1,200.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because the electronic patent may issue shortly after the issue fee is paid, any desired continuing application is preferably filed prior to payment of the issue fee so as to insure copendency with its parent application.**

CURRENT CORRESPONDENCE ADDRESS

(Note: Use Block 1 for any change of address)

23373

SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, NW, SUITE 9000
WASHINGTON, DC 20006-1811

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s)

Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)

(Signature)

(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	Undiscounted	\$1200	\$0	\$0	\$1200	03/20/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVID J STEADMAN	1656	435-023000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/ALA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Sughrue Mion, PLLC

2. _____

3. _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

SEIKAGAKU CORPORATION

Chiyoda-ku, Tokyo, Japan

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment (Please first reapply any previously paid fee shown above):

- ☒ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed Check ☐ Non-electronic payment by credit card (Attach form PTO-2038)
☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 19-4880

5. Change of Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29.
☐ Applicant asserting small entity status. See 37 CFR 1.27.
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /markboland/

Date December 21, 2023

Typed or Printed Name Mark Boland

Registration No. 32,197



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

23373 7590 12/20/2023
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

EXAMINER

STEADMAN, DAVID J

ART UNIT

PAPER NUMBER

1656

DATE MAILED: 12/20/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	03/20/2024

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23373 7590 12/20/2023
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	03/20/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
STEADMAN, DAVID J	1656	435-023000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

1 _____

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via the USPTO patent electronic filing system ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

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NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959
23373 7590 12/20/2023 SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 9000 WASHINGTON, DC 20006			EXAMINER STEADMAN, DAVID J	
			ART UNIT	PAPER NUMBER
			1656	
DATE MAILED: 12/20/2023				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).

<https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf>

Routine uses of the information in this record may include disclosure to:

- 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
- 2) a federal, state, local, or international agency, in response to its request;
- 3) a contractor of the USPTO having need for the information in order to perform a contract;
- 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
- 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
- 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
- 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
- 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
- 9) the Office of Personnel Management (OPM) for personnel research purposes; and
- 10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/346,420	Applicant(s) MIZUMURA et al.	
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to applicant's response filed on November 24, 2023.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-10 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a) ☒ All b) ☐ Some* c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☒ Certified copies of the priority documents have been received in Application No. 14/650,767 .
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.	6. ☐ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.	7. ☐ Other _____.
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date. <u>20231206</u> .	

/David Steadman/ Primary Examiner, Art Unit 1656	
---	--

NOTICE OF ALLOWABILITY

Status of the Application

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Applicant's amendment to the claims, filed on November 24, 2023, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's submission of a terminal disclaimer, filed on November 24, 2023, is acknowledged.

Applicant's remarks and declaration under 37 CFR 1.132 filed on November 24, 2023 in response to the non-final rejection mailed on September 15, 2023 have been fully considered.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Terminal Disclaimer

The terminal disclaimer filed on October 10, 2023 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. Patent Nos. 10,144,923, 10,982,202, and 11,236,318 and any patent granted on Application Number 17/554,128 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Examiner's Amendment to the Claims

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Hui Wauters on December 6, 2023.

Please replace the claims filed on November 24, 2023 with the following claim set.

1. (Currently Amended) A method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C ~~having activity of Factor C~~ ~~secreted~~ in the culture supernatant, wherein the recovered horseshoe Factor C has activity of Factor C, and wherein the horseshoe crab Factor C is a *Tachypleus tridentatus* Factor C or a *Limulus polyphemus* Factor C,

expressing the horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,

expressing the horseshoe crab Pro-clotting enzyme in a mammalian cell as a host cell,
and

producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,

wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.

2. (Original) The method according to claim 1, wherein the mammalian cell expressing the Factor B is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

3. (Original) The method according to claim 1, wherein the mammalian cell expressing the Pro-clotting enzyme is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

4. (Currently Amended) The method according to claim 1, wherein the horseshoe crab for the Factor B and/or the horseshoe crab for the Pro-clotting enzyme is selected from the group consisting of *Tachypleus tridentatus* and *Limulus polyphemus*.

5. (Original) The method according to claim 1, wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

6. (Original) The method according to claim 1, wherein the horseshoe crab Factor C has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a nonreducing condition.

7. (Original) The method according to claim 1, further comprising filtering the culture supernatant comprising the horseshoe crab Factor C.
8. (Currently Amended) The method according to claim 1, wherein the endotoxin assay agent is formulated as a solid, a liquid, or a gel~~horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are mixed in a formulation.~~
9. (Currently Amended) The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are ~~formulated separately~~ expressed in individually established host cells.
10. (Currently Amended) A method for measuring endotoxin in a test specimen, the method comprising
expressing a horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C in the culture supernatant,
wherein the recovered horseshoe Factor C has activity of Factor C, and wherein the horseshoe crab Factor C is a *Tachypleus tridentatus* Factor C or a *Limulus polyphemus* Factor C,
expressing a horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,
expressing a horseshoe crab Pro-clotting enzyme in a mammalian cell as a host cell,

producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,
~~a step of mixing the[[an]] endotoxin assay agent produced by the method according to claim 1 and a test specimen, and~~
~~a step of measuring [[a]] progress of a cascade reaction,~~
wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.

Claim Objections

The objection to claim 4 is withdrawn in view of the applicant's instant amendment to delete the comma from the phrase "*Tachypleus tridentatus*, and *Limulus polyphemus*".

Claim Rejections - 35 USC § 112(b)

The rejection of claim 9 is withdrawn in view of the examiner's amendment to recite "wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are separately expressed in individually established host cells".

Claim Rejections - 35 USC § 112(d)

The rejection of claim 8 is withdrawn in view of the examiner's amendment to recite "wherein the endotoxin assay agent is formulated as a solid, a liquid, or a gel".

Claim Rejections - 35 USC § 103

The rejection of claims 1-10 under 35 U.S.C. 103 as being unpatentable over Ding-1 et al. (US 5,858,706; cited on the IDS filed on July 3, 2023) in view of Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on July 3, 2023) is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration under 37 CFR 1.132 (hereafter "declaration"), and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *Biotechnol. Lett.* 23:71-76, 2001; filed on November 17, 2023) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

The rejection of claims 1-10 under 35 U.S.C. 103 as being unpatentable over Ding et al. (US 5,716,834; cited on the IDS filed on July 3, 2023) in view of Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian

host cell COS-1, however, the recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

Claim Rejections - Double Patenting

The rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claim 8 of U.S. Patent No. 10,982,202 B2 (Cite No. 9 of the IDS filed on July 3, 2023) in view of Tamura is withdrawn in view of the applicant's submission of a terminal disclaimer disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. Patent No. 10,982,202.

The rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 8, and 9 of U.S. Patent No. 10,144,923 B2 (Cite No. 2 of the IDS filed on July 3, 2023) in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the

recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

The rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claims 2, 4, and 10-12 of U.S. Patent No. 11,236,318 B2 (Cite No. 10 of the IDS filed on July 3, 2023) in view of in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's submission of a terminal disclaimer disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. Patent No. 11,236,318.

The rejection of claims 1 and 3-10 on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,040,254 B2 (cited on the Form PTO-892 mailed on September 15, 2023) in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the recombinant Factor C was not secreted and remained insoluble within the host cell,

indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

The rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claim 1 and 7-9 of U.S. Patent No. 11,499,177 B2 (cited on the Form PTO-892 mailed on September 15, 2023) in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

The provisional rejection of claims 1-6, 8, and 9 on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending

application 17/554,128 and the provisional rejection of claims 7 and 10 on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of the reference application in view of Tamura is withdrawn in view of the applicant's submission of a terminal disclaimer disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of any patent granted on Application Number 17/554,128.

The provisional rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claims 1-3 of co-pending application 17/272,805 in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

The provisional rejection of claims 1-10 on the ground of nonstatutory double patenting as being unpatentable over claims 1 and 7-9 of co-pending application

18/045,705 in view of Ding-1, Ding-2, and Tamura is withdrawn in view of the applicant's instant amendment to the claims, the applicant's declaration, and the applicant's remarks. The evidence presented in the declaration shows that Factor C as prepared by the method of Ding-1 exhibits no Factor C activity. Also, the applicant's previously presented evidence (Wang et al., *supra*) teaches expression of recombinant Factor C using the mammalian host cell COS-1, the recombinant Factor C was not secreted and remained insoluble within the host cell, indicating that the native signal sequence was not able to direct full-length recombinant Factor C out of the cell (p. 71, paragraph bridging columns 1-2). However, the applicant's declaration shows that Factor C prepared according to the claims is secreted into the culture medium and exhibits Factor C activity, which is unexpected given the evidence of record and rebuts a *prima facie* case of obviousness.

Information Regarding Quick Path IDS Program

For situations when the applicant needs to file an IDS after the issue fee has been paid, The United States Patent and Trademark Office (USPTO) has implemented a Quick Path Information Disclosure Statement (QPIDS) program intended to reduce pendency and applicant costs. This pilot program will permit an examiner to consider an IDS after payment of the issue fee without the need to reopen prosecution, effectively obviating the need to pursue a Request for Continued Examination (RCE). Where the examiner determines that no item of information in the IDS necessitates reopening prosecution, the Office will issue a corrected notice of allowability. In addition to reducing pendency, this program will promote efficiency in the examination process.

There will be no fee required to use this program, beyond existing fees, *e.g.*, fees for IDS submission. More information on this and other new initiatives at the Patent Office can be found at http://www.uspto.gov/patents/init_events/patapp-initiatives-timeline.jsp or by calling the examiner or his supervisor.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656

<i>Examiner-Initiated Interview Summary</i>	Application No. 18/346,420	Applicant(s) MIZUMURA et al.		
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA (First Inventor to File) Status Yes	Page 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	Title	Type
DAVID J STEADMAN	Primary Examiner	Telephonic
HUI WAUTERS	Attorney of Record	

Date of Interview: 06 December 2023

Issues Discussed:

Proposed Amendment(s)

A proposed examiner's amendment was presented to the applicant's representative on December 5, 2023 and was authorized for entry on December 6, 2023.

/David Steadman/ Primary Examiner, Art Unit 1656	
<p>Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04</p> <p>Please further see: MPEP 713.04 Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b) 37 CFR § 1.2 Business to be transacted in writing</p>	

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

CPC						
Symbol					Type	Version
C12N	/	9	/	6408	F	2013-01-01
G01N	/	33	/	579	I	2013-01-01
C12Y	/	304	/	21069	I	2013-01-01
G01N	/	33	/	56911	I	2013-01-01
C12Y	/	304	/	21084	I	2013-01-01
G01N	/	2333	/	96411	A	2013-01-01

CPC Combination Sets					
Symbol		Type	Set	Ranking	Version
	/				

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 10	
/David Steadman/ Primary Examiner, Art Unit 1656 (Primary Examiner) _____ (Date) 07 December 2023		O.G. Print Claim(s) 1	O.G. Print Figure NONE

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

INTERNATIONAL CLASSIFICATION			
CLAIMED			
C12N	/	9	/ 64
C12Q	/	1	/ 37
G01N	/	33	/ 579
NON-CLAIMED			
	/		/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 10	
/David Steadman/ Primary Examiner, Art Unit 1656 (Primary Examiner) _____ (Date) _____		07 December 2023 O.G. Print Claim(s) 1	O.G. Print Figure NONE

Issue Classification 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47															
CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 10	
/David Steadman/ Primary Examiner, Art Unit 1656 (Primary Examiner) _____ (Date) 07 December 2023		O.G. Print Claim(s) 1	O.G. Print Figure NONE

<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

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<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

Search Notes		
Search Notes	Date	Examiner
PATENT DATA PORTAL inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
SEARCH inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
Reviewed prosecution history for priority applications 14/650,767, 15/983,725, 16/399,575, 16/399,386, 17/554,128	09/12/2023	/DJS/
SEARCH, STN database keyword search, see attached search printouts for search strategy and databases searched	09/12/2023	/DJS/
GOOGLE keyword search, see attached search printouts for search strategy	09/12/2023	/DJS/
Reviewed Global Dossier for applications EP13862466, EP18190686, and EP19216609	09/12/2023	/DJS/
C12N9/6408, C12Y304/21084 with keyword search, see SEARCH printout	09/12/2023	/DJS/
PATENT DATA PORTAL inventor name, applicant, and assignee search, all inventors searched	12/07/2023	/DJS/
PE2E SEARCH inventor name, applicant, and assignee search, all inventors searched	12/07/2023	/DJS/
Updated PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	12/07/2023	/DJS/
Updated C12N9/6408, C12Y304/21084 with keyword search, see PE2E SEARCH printout	12/07/2023	/DJS/

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	PE2E SEARCH database keyword search, see attached search printout for search strategy and databases searched	12/07/2023	/DJS/

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Bibliographic Data

Application No: 18/346,420

Foreign Priority claimed: ☒ Yes ☐ No

35 USC 119 (a-d) conditions met: ☒ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged:

/David Steadman/

Examiner's Signature

Initials

Title:

NOVEL RECOMBINANT FACTOR C AND METHOD FOR
PRODUCING THE SAME, AND METHOD FOR MEASURING
ENDOTOXIN

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/03/2023	435	1656	Q289014
RULE			

APPLICANTS

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Toshio Oda,

Shun-Ichiro Kawabata, Fukuoka, JAPAN

CONTINUING DATA

This application is a CON of 17554128 12/17/2021

17554128 is a CON of 16399386 04/30/2019 PAT 11236318

16399386 is a CON of 15983725 05/18/2018 PAT 10982202

15983725 is a CON of 14650767 06/09/2015 PAT 10144923

14650767 is a 371 of PCT/JP2013/083082 12/10/2013

FOREIGN APPLICATIONS

JAPAN JP2012-269840 12/10/2012

IF REQUIRED, FOREIGN LICENSE GRANTED**

07/27/2023

STATE OR COUNTRY

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FILING FEE RECEIVED

\$6,160

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L5	7687	(horseshoe adj crab) OR limulus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 09:46 AM
L6	13415	factor adj "c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 09:47 AM
L7	13415	"factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 09:47 AM
L8	335	L5 same L6	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 09:47 AM
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L12	9	L10 and ((chinese adj hamster) OR CHO)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:02 AM
L13	2	L10 and (hek OR (embryonic adj kidney))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:02 AM
L14	6	L10 and (hek\$ OR (embryonic adj kidney))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:03 AM
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L16	2	"6,645,724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:04 AM
L17	12	"2012118226"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 10:35 AM
L18	4	"5716834".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/12 03:58 PM
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L20	18057	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2016/09/13 09:23 AM

			JPO; DERWENT)				
L21	20466	L19 or L20	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
L22	91	L21 and (horseshoe adj crab)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
L23	66	L21 and tridentatus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:25 AM
L24	41	L21 and rotundicauda	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:26 AM
L25	108	L22 or L23 or L24	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:26 AM
L26	40	L25 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 09:26 AM
L27	23	("5,858,706" OR "5,712,144" OR "5,795,962" OR "6,077,946" OR "20090208995" OR "20030054432" OR "20040235080" OR "20140024062").pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2016/09/13 11:34 AM
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L29	4	"5,858,706".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 11:27 AM
L30	2	"6,645,724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 03:42 PM
L31	2	"20140249082".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 04:45 PM
L32	2474	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:07 PM
L33	18751	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:07 PM
L34	21180	L32 or L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:07 PM
L35	19	L33 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM

L36	13	L35 and "factor C"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM
L37	2	L36 and (sialic OR sialyl\$)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/04/06 05:08 PM
L38	14664	"factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:53 PM
L39	218	L38 and (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:53 PM
L40	7	L38 same (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L41	218	L39 and (cho OR "chinese hamster ovary")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L42	805	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:54 PM
L43	2	L42 and (dg44 OR dg- 44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L44	5676	(cho OR "chinese hamster ovary") same (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L45	211	L44 and L38	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:55 PM
L47	7	L45 and horseshoe	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/30 03:56 PM
L48	20	horseshoe and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:45 AM
L49	218	"factor c" and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:47 AM
L50	211	L49 not L48	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:47 AM
L51	0	L50 and horseshoe	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:48 AM
L52	2	"factor c".clm. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:48 AM
L53	6	"factor c".ti. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM

			JPO; DERWENT)				
L54	7	"factor c".ab. and (dg44 OR dg-44 OR dg/44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM
L55	1	L54 not L53	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM
L56	2	"9133495".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:36 PM
L57	19132	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L58	6	L57 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L59	2485	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L60	3	L59 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L61	7	L58 or L60	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L62	19414	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L63	6	L62 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L64	2493	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L65	3	L64 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L66	7	L63 or L65	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L67	19635	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L68	6	L67 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L80	3	("20080131929" "20100086967" "20130295613").PN.	(US-PGPUB; USPAT)	OR	OFF	OFF	2018/09/07 10:58 AM
L81	19653	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM

			JPO; DERWENT)				
L82	6	L81 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM
L94	6	"10144923".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 09:23 AM
L96	6	(US-20080131929-\$ US-20100086967-\$ US-20130295613-\$).DID.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 10:53 AM
L97	20399	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L98	40	L97 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L99	23093	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:15 AM
L100	95	L99 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L101	54	L100 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L102	24	L101 and ("chinese hamster" OR CHO OR human OR HEK)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L103	627	tridentatus OR rotundicauda	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L104	389	L103 and endotoxin	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L105	263	L104 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L106	224	L103 same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L107	216	L105 and L106	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L108	140	L107 and "37"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L109	1050017	"2 hours"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L110	114	L108 and L109	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM

			USOCR; FPRS; EPO; JPO; DERWENT)				02:42 PM
L113	349173	cascade	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:44 PM
L114	169	L107 and L113	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:45 PM
L115	468	limulus adj test	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:30 PM
L116	1	L115 same cation	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:31 PM
L117	3	"6,645,724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 12:19 PM
L118	13	("20030054432" "20040 235080" "20080131929 " "20090208995" "2010 0086967" "2013029561 3" "20140024062" "201 40249082" "5712144" "5 716834" "5795962" "5 858706" "6077946").PN .	(US-PGPUB; USPAT)	OR	OFF	OFF	2020/04/22 02:32 PM
L119	23218	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L120	85	L119 AND (tridentatus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L121	54	L120 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:38 PM
L122	33	L121 and endotoxin.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:39 PM
L123	23351	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L124	95	L123 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L125	55	L124 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L126	10	L125 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L127	8	L125 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2020/06/24 03:12 PM

			JPO; DERWENT)				
L128	10	L126 or L127	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:12 PM
L129	23458	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L130	98	L129 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L131	57	L130 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L132	11	L131 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L133	9	L131 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L134	11	L132 or L133	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L135	753	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:52 AM
L136	44	L135 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L137	829430	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L138	104016	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L139	14	L136 and (L137 OR L138)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L140	4	"6645724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L141	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L142	46	L141 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L143	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L144	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2021/03/04 04:49 AM

			JPO; DERWENT)				
L145	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L146	46	L145 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L147	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L148	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L149	15	L146 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L150	64	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L151	24	L150 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L152	886720	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L153	112690	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L154	69	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L155	19	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L156	812	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L157	50	L156 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L158	16	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L159	40	L156 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:30 AM
L160	13	(L152 OR L153) same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:31 AM
L161	204	(L152 OR L153) same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2021/10/18 08:40 AM

			JPO; DERWENT)				
L162	13	L160 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:41 AM
L163	4875	(hek OR hek293 OR (human adj embryo\$)).clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L164	8	L157 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L165	14	L154 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L166	6	L165 not L164	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L167	8	hek293 same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:50 AM
L168	24311	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L169	103	L168 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L170	63	L169 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L171	15	L170 and (L152 or L153 OR hek293)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:52 AM
L172	2183	ding-J\$.in.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L173	22336	"factor c"	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L174	26	172 AND 173	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L175	722713	secret\$	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L176	13	174 AND 175	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L177	563	tachypleus	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L178	172826	ding-J\$.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2023/03/13 03:56 PM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L179	81001	"factor c"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:56 PM
L180	117	178 AND 179	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L181	2156	tachypleus	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM

L182	17	180 AND 181	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L183	3284055	secret\$	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L184	13	182 AND 183	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L185	93965	"factor b"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO,	OR	ON	ON	2023/03/14 09:54 AM

			MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L188	438	185 AND 177	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:55 AM
L189	3617370	"chinese hamster" OR CHO????	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM
L190	104	188 AND 189	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM
L191	21	recombinant?? adj5 185	(US-PGPUB; USPAT;	OR	ON	ON	2023/03/14

			USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				10:04 AM
L192	12	("0000001" OR "10144923" OR "20020068325" OR "20030054432" OR "20040235080" OR "20080131929" OR "20090208995" OR "20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5795962").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/14 05:22 PM
L193	21	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 05:59 PM
L194	115	L156 AND (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L195	10351	limulus OR tachypleus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L196	25	194 AND 195	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L197	9	196 NOT 193	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L198	2271	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:49 AM
L199	25141	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L200	27	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L201	379035	(human ADJ embryo\$) OR hek???	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY,	OR	ON	ON	2023/03/15 05:51 AM

			CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L202	3817922	189 OR 201	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:51 AM
L203	37	200 AND 202	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:52 AM
L204	28	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/11 03:26 PM
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		"20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5712144" OR "5716834" OR "5795962" OR "5840510" OR "5858706" OR "6077946" OR "6645724").pn.					
L206	1	("6733997").pn.	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/09/11 04:10 PM
L207	22	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:10 AM
L208	24	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM
L209	29	207 OR 208	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM
L210	26980	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as,aanm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:33 AM
L211	29	198 AND 210	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:34 AM
L212	22825	((human ADJ embryo\$) OR hek???).clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:34 AM
L213	368450	("chinese hamster" OR CHO????).clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE,	OR	ON	ON	2023/12/07 05:35 AM

			SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L214	18	211 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:35 AM
L215	10356	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	OFF	OFF	2023/12/07 05:40 AM
L216	177	215 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:41 AM
L217	174705	horseshoe	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY,	OR	ON	ON	2023/12/07 05:41 AM

			CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L218	25	216 AND 217	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:41 AM
L219	54	L145 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:47 AM
L220	34	L154 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:48 AM
L221	55	219 OR 220	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/12/07 05:48 AM
L222	23	221 AND (212 OR 213)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/12/07 05:48 AM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q289014

Hikaru MIZUMURA, et al.

Appln. No.: 18/346,420

Group Art Unit: 1656

Confirmation No.: 6959

Examiner: DAVID J STEADMAN

Filed: July 3, 2023

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Hikaru MIZUMURA, hereby declare and state:

I am a citizen of Japan;

I have received the degrees of Bachelor of Science in 1993, Master of Science in 1996,
and Doctor of Philosophy (Ph. D.) in 1999 from Saitama University;

I was employed by RIKEN (the Institute of Physical and Chemical Research) from April
1, 1999 to March 31, 2002 as Special Postdoctoral Researcher of Laboratory of Cellular and
Molecular Biology, with responsibility for research on endonuclease from yeast mitochondria;

I was employed by Tokai University School of Medicine from April 1, 2002 to March 31,
2005 as Postdoctoral Researcher of Department of Molecular Neuroscience, with responsibility
for research on biochemical characterization of ALS2 protein;

I was employed by The University of Tokyo from April 1, 2005 to August 31, 2007 as Postdoctoral Researcher of Laboratory of Microbiology, with responsibility for research on DNA replication in virulence bacteria *Staphylococcus aureus*;

I have been employed by Seikagaku Corporation since September 1, 2007, where I hold a position as head of LAL Research, Central Research Laboratory, with responsibility for research and development of reagents for measuring endotoxin and/or glucan by using horseshoe crab proteins; and

I am the first-named inventor of the above-identified application; I am familiar with the subject matter of the above-identified application and the Office Action mailed September 15, 2023 in the above-identified application.

This Declaration is submitted to show unexpected effects provided by our invention over the cited references, Ding et al. (US 5,858,706, “Ding-1”), Tamura et al. (US 2009/0208995 A1); Ding et al. (US 5,716,834, “Ding-2”), thus establishing patentability of our invention over the cited references.

The following additional experiments were conducted by me or under my direct supervision.

Factor C Activity in Cell Body Fraction of CHO and HEK cells Expressing Factor C

(1) Expression of LpFC and TtFC in CHO and HEK Cells

In reference to “(2) *Expression of Factor C in Mammalian Cells (CHO DG44)*” sections of the present specification, and WO2018/074498A1, Factor C of *Limulus polyphemus* (LpFC) and *Tachypleus tridentatus* (TtFC) were recombinantly expressed in CHO DG44, CHO-K1, CHO-S and HEK293 cells.

(2) Confirmation of Intracellular Factor C

The cell bodies of CHO-K1, CHO DG44, CHO-S, and HEK293 transformed so as to express LpFC or TtFC were solubilized at room temperature with Sample Buffer Solution without Reducing Reagent for SDS-PAGE (6x) (Nacalai tesque, Japan). The solubilized cells were subjected to SDS-PAGE without purification and subsequent western blotting using anti-Factor C antibody to confirm the presence of intracellular Factor Cs.

As shown in Figure 1, A and B, and Figure 2 below, intracellular Factor Cs were confirmed to be present in CHO-K1, CHO DG44, CHO-S, and HEK cells transformed so as to express LpFC or TtFC.

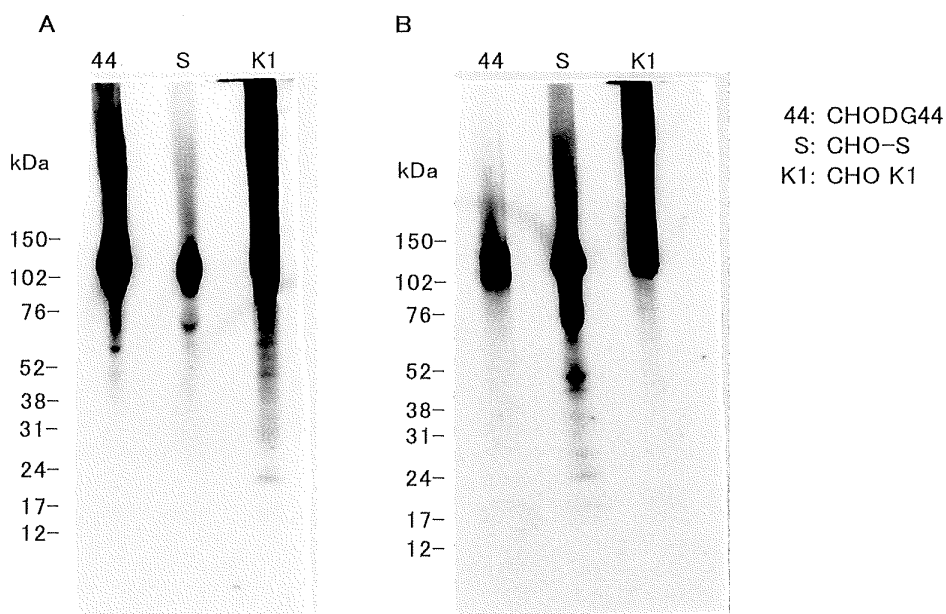


Figure 1: Intracellular LpFC (A) and TtFC (B) of transformed CHO cells; in some samples, the band of Factor C broadened probably due to the impurities arising from cell debris.

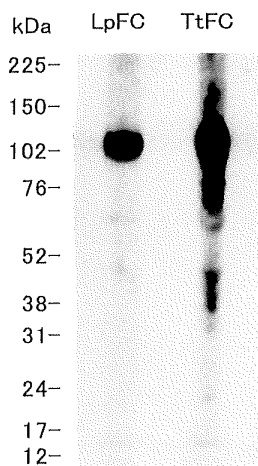


Figure 2: Intracellular LpFC and TtFC of transformed HEK cell; the band of TtFC broadened probably due to the impurities arising from cell debris.

(3) Measurement of Factor C Activity

Test samples for measuring Factor C activity of intracellular Factor C were prepared according to the procedure disclosed in EXAMPLE 2 of US 5,858,706 (Ding-1). Specifically, cell pellet collected by centrifugation was washed with phosphate buffered saline, suspended in breaking buffer (1 w/v % sodium dodecyl sulfate (SDS) in 0.05 M Tris-HCl, pH 8.0, containing 0.1 M NaCl), and solubilized overnight at 4 °C by rotation on an angular rotator. The lysate was cleared by centrifugation, and subjected to membrane filtration (Amicon Ultra-0.5mL Ultracel 30K or Vivaspin 500 MWCO 30,000) for the removal of residual SDS. The retentate was used as cell body fraction (cell lysate) for the following procedures.

Factor C activity was measured in reference to a method disclosed in “4. *Activity Measurement*” section of the present specification. The assay was conducted without endotoxin (blank) or under the presence of 0.1 EU/ml of endotoxin. Factor C activity was calculated as a

difference of absorbance change rate between the measured value under the presence of 0.1 EU/ml of endotoxin and blank value. The culture supernatants of CHO, and HEK cells were used as positive controls. The results are summarized in Tables 1 and 2 below.

As shown in Tables 1 and 2, Factor C activity of intracellular TtFC or LpFC was not detected in the samples prepared as in US 5,858,706 (Ding-1).

Table 1: LpFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μL)	ΔmAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.1
		4	0.0
	Culture supernatant	1	1.1
		2	16.1
		4	16.2
CHO-S	Cell lysate	1	-0.1
		2	0.1
		4	0.1
	Culture supernatant	1	2.5
		2	9.5
		4	12.9
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	11.2
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	7.3

Table 2: TtFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μL)	ΔmAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	0.3
		2	1.8
		4	6.4
CHO-S	Cell lysate	1	0.0
		2	-0.1
		4	0.0
	Culture supernatant	1	0.2
		2	8.5
		4	14.5
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	14.3
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	9.7

$\Delta mAbs/min = [mAbs/min \text{ at } 0.1 \text{ EU/ml}] - [mAbs/min \text{ at } 0 \text{ EU/ml}]$
[mAbs/min at 0.1 EU/ml]: Absorbance change rate at 0.1 EU/ml of Endotoxin concentration
[mAbs/min at 0 EU/ml]: Absorbance change rate without Endotoxin

As seen from the above data, while no Factor C activity was observed in the intracellular fraction prepared based on EXAMPLE 2 of US 5,858,706 (Ding-1), excellent Factor C activity was observed in the culture supernatant as described in the Examples and Fig. 6 of the present application. These results demonstrate superior effects provided by the claimed feature of "secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the culture supernatant." The superior effects provided by our invention are unexpected from Ding-1, Tamura et al. and Ding-2.

DECLARATION UNDER 37 C.F.R. § 1.132
Appln. No.: 18/346,420

Attorney Docket No.: Q289014

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 22 November 2023

Hikaru Mizumura
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Recombinant COS-1 cells express Carcinoscorpius rotundicauda Factor C

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The cDNA encoding Carcinoscorpius rotundicauda Factor C, CrFC, when expressed in mammalian cells, produced full length and truncated variants of rFC. In an *in vitro* coupled transcription-translation system, aberrant products were generated from pCDNAI/CrFC21, as opposed to pCIneo/CrFC21 which gave rise to the expected polypeptide. COS-1 cells transfected with pCIneo/CrFC21 expressed Factor C-related proteins which differ from those produced in the yeast, *Pichia pastoris*. The expression of a complex, mosaic protein such as the rFC in mammalian cells also resembled other mammalian blood factors, indicating that such cells could be used for production of a second generation 'limulus lysate'.

Introduction

The defense mechanism of the horseshoe crab against invading pathogens consists of an amoebocyte-mediated coagulation cascade. Trace amounts of lipopolysaccharide (LPS) from the Gram-negative bacterial cell wall activates the key enzyme, Factor C (FC), which initiates the cascade to result in a gel clot (Nakamura *et al.*, 1986). Extremely sensitive gelation (Ho, 1983) and colorimetric assays (Miura *et al.*, 1992) are available for the detection of activated FC. This forms the basis for the Limulus amoebocyte lysate (LAL) test that is widely used in pharmaceutical industry for endotoxin detection. However, overharvesting of the horseshoe crabs has led to their near-extinction in recent years. Towards obtaining a second generation 'limulus lysate', Factor C from the Southeast Asian horseshoe crab, *Carcinoscorpius rotundicauda* (CrFC), has been studied extensively. A single chain form of 132 kDa, as well as a double chain form of 80 and 52 kDa have been purified to homogeneity (Ding *et al.*, 1993). Two full length cDNAs, CrFC26 of 4182 bp and CrFC21 of 3448 bp have been cloned and characterised (Ding *et al.*, 1995). These two cDNAs differ mainly in the length and nucleotide composition of their 5' and 3' untranslated regions and have a 99.1% homology in the coding region. The CrFC26 and 21 as well as their truncated forms have been studied in *in vitro* coupled transcription-translation (TnT) assays using rabbit reticulocyte lysate (Roopashree *et al.*, 1995). These assays generated the expected translated products with full-length CrFC21 constructs, thus forming the basis of our choice of this cDNA species for expression in heterologous systems.

Being endotoxin-free, the methylotrophic yeast, *Pichia pastoris* was used to produce high amounts of CrFC transcripts, as well as recombinant Factor C (rFC) that was possibly membrane bound (Roopashree *et al.*, 1996).

Other comparable serine proteases such as Factors V, VII, IX, X, and XII (Citarella *et al.*, 1992), and the human tissue type plasminogen activator (t-PA), with structural organisations similar to CrFC have been expressed in mammalian cells. Expression of complex proteins in lower eukaryotic yeast systems may differ from higher eukaryotes, as reported in the case of both Factor XII and t-PA (Citarella *et al.*, 1992; Philipova *et al.*, 1992 and Lemmont *et al.*, 1985, respectively). Accordingly, expression of CrFC in mammalian cells such as COS-1 would offer the advantages of all post-translational modifications that could be offered in a heterologous system. It would be of immense interest to study the expression of an invertebrate 'complement like' factor in mammalian cells, such as COS-1. These studies would also determine the feasibility of establishing a permanent cell line that can produce rFC.

Materials and methods

Construction of recombinant expression vectors (Figs 1 and 2)

All cloning procedures were carried out according to Sambrook *et al.* (1989). Unless co-transfected with a second plasmid encoding a gene for drug resistance, the vector pCDNAI is only designed for transient expression. On the other hand, cells transfected with pCIneo

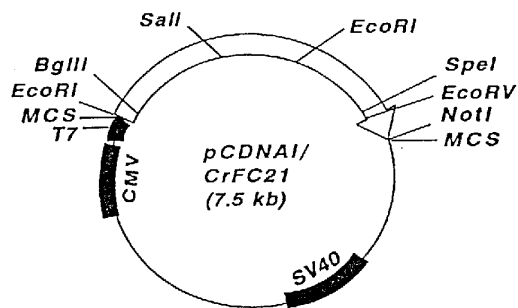


Figure 1 Recombinant construct pCDNAI/CrFC21. The vector pCDNAI (Invitrogen, USA) was first digested with EcoRI and NotI, to receive the 1.1 kb fragment of CrFC21 flanked by EcoRI and NotI (Roopashree et al., 1995), generating the intermediate construct pCDNAI/CrFC21EN. This plasmid was then linearised with EcoRI and ligated with the remaining 2.316 kb EcoRI flanking fragment of CrFC21, to create the complete recombinant vector pCDNAI/CrFC21.

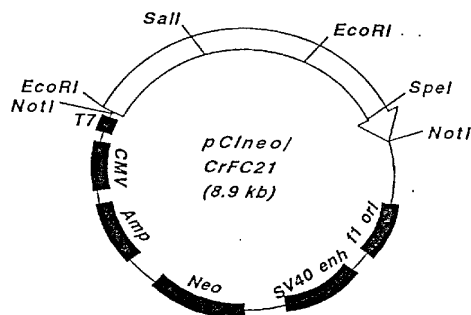


Figure 2 Recombinant construct pCIneo/CrFC21. The parent vector pCIneo was digested with NotI, and treated with calf intestinal phosphatase to remove 5' phosphate groups. It was then ligated with full length CrFC21 flanked by NotI ends, derived from an intermediate plasmid construct, pBluescriptIIISK/CrFC21. The pCIneo/CrFC21 3'-5' construct containing CrFC21 in the reverse orientation was used as a negative control for subsequent TnT and transfection studies.

also express neomycin phosphotransferase, and can be used for selection of stably expressing transfectants, using the antibiotic G-418.

The vectors for expression of CrFC in mammalian cells carry the cytomegalovirus (CMV) immediate early promoter/enhancer region to promote constitutive expression. They also contain the SV40 early promoter region for induction of transient, episomal replication in cells such as COS-1 or COS-7, expressing the SV40 large T antigen (Gluzman, 1981). The CrFC21 insert

in both constructs contains the native ATG start codon, along with its Kozak consensus element.

In vitro coupled transcription-translation

The recombinant constructs were subjected to T7 promoter driven TnT reactions (Promega, USA), and products analysed as described earlier (Roopashree et al., 1995). In this work, [³H]-leucine (Amersham, UK) was used as the tracer.

Transfection of COS-1 cells

COS-1 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% foetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 10 mM HEPES. CrFC recombinant constructs were isolated using cesium chloride-ethidium bromide gradients (Sambrook et al., 1989). Transfection was carried out by electroporation (Buckler, 1991). Briefly, 10⁷ cells were trypsinised, washed and resuspended in 0.4 ml of PBS, incubated on ice for 10 min with 20–40 µg of DNA and electroporated at 1.2 kV at 25 µF in a cuvette of 0.4 cm path (Gene Pulser, Biorad).

SDS-PAGE and Western analyses

Cells were harvested at 48 and 72 h post-transfection. Cell lysates were prepared by three rounds of freeze-thawing. Fifty µg of the lysate protein was subjected to reducing SDS-PAGE. Gels were electrotransferred to Immobilon PVDF membrane (Millipore), probed with rabbit anti-Factor C antibody, followed by goat anti-rabbit antibody conjugated with horseradish peroxidase, and developed with the substrate mixture of 0.03% 4-chloronaphthol and 3% H₂O₂.

Results and discussion

TnT with pCDNAI/CrFC21 and pCIneo/CrFC21 yielded variable rFCs

The presence and orientation of the full-length CrFC21 insert in pCDNAI/CrFC21 and pCIneo/CrFC21 were verified firstly by restriction analysis, secondly by Southern hybridisation using ³²P-CrFC21 cDNA probe (data not shown), and thirdly by PCR amplification of the CrFC21 fragment from the insert (Roopashree et al., 1996).

Further confirmation of the expression competency was carried out in a TnT assay, before these constructs were utilised for transfection studies. Analysis of the *in vitro* TnT products on SDS-PAGE, followed by fluorography, revealed that pCIneo/CrFC21 was capable of generating the expected polypeptide corresponding to a molecular mass of 113 kDa (Fig. 3a).

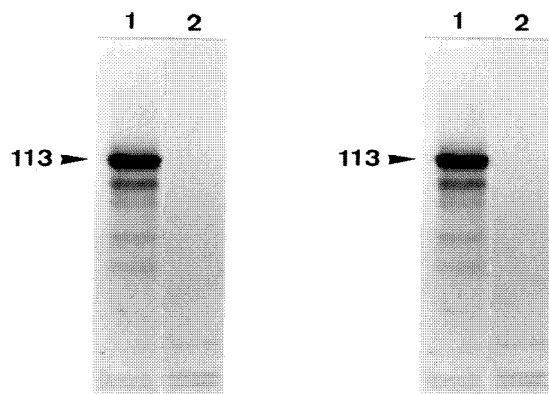


Figure 3 Autoradiograms of TnT products from CrFC21 expression vectors. ^3H -leucine labelled TnT products from (a) pCIneo/CrFC21, and pCIneo/CrFC213'-5' (lanes 1 & 2, respectively) and (b) pCDNAI and pCDNAI/CrFC21 (lanes 1 & 2 respectively). Sizes are indicated in kDa. As expected, the controls pCDNAI and pCIneo/CrFC213'-5' did not generate any product. The minor lower molecular weight bands generated by pCIneo/CrFC21 could be breakdown products of the 113 kDa product. Such an occurrence of breakdown products has also been observed with the Luciferase control DNA that was used to monitor the reaction.

In contrast, the TnT reaction utilising pCDNAI/CrFC21 gave rise to a major product of only 32 kDa, and a minor product of 84 kDa (refer Fig. 3b). Since restriction analysis, Southern hybridisation and PCR have confirmed the presence of full length CrFC21 in pCDNAI, these truncated TnT products could be due to either the recognition of internal start codons at M²³⁸² and M⁶⁶⁶ (Ding *et al.*, 1995), respectively, or the introduction of premature stop codons, following nonspecific point mutations in the insert. These studies have further proven the importance of *in vitro* experiments to verify the integrity of the cDNAs in expression vectors, before further cloning into eukaryotic hosts for *in vivo* expression.

pCIneo/CrFC21-transfected COS-1 cells expressed full length and truncated variants of rFC

COS-1 cells were transfected with recombinant vectors pCIneo/CrFC21, pCIneo/CrFC213'-5' (reverse orientation of insert) and pCDNAI/CrFC21. Transfectants were analysed for expression of rFC at 48 and 72 h post transfection. Cells transfected with pCDNAI/CrFC21 failed to generate rFC. This observation concurred with the earlier TnT assay which generated a major aberrant product of 32 kDa with this construct (Fig. 3).

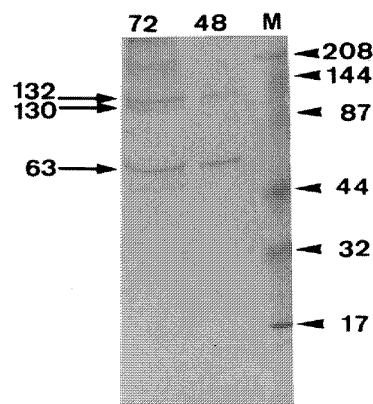


Figure 4 Immunoblot of rFC. The rFC derived from COS-1 cells at 48 and 72 h post transfection, as detected by anti-FC antibodies. The sizes of prestained kaleidoscope (Biorad) molecular weight markers (lane M) are indicated in kDa.

Immunoblots treated with anti-Factor C antibodies revealed that cells transfected with pCIneo/CrFC21 indeed expressed rFC (Fig. 4).

Cells harvested at 48 h post transfection showed the presence of an rFC related protein of 132 kDa, corresponding to full length CrFC21. On the other hand, those harvested at a later time point of 72 h showed the presence of an additional band of 130 kDa, resulting in a closely migrating doublet of rFC proteins. In addition, at both these time points, transfectants also expressed a third band corresponding to an rFC-related protein of 63 kDa.

The presence of rFC proteins of different molecular masses in COS-1 cells indicated that the 132/130 kDa doublet could be a mixture of glycosylated/unglycosylated variants. Such heterogeneity in the recombinant products has been reported in several cases. For example, two electrophoretic variants of murine interleukin-4 were expressed in COS-7 cells that were present as a closely spaced doublet with a molecular weight of 19 kDa (Ramanathan *et al.*, 1989). The same observation has also been reported in the case of human recombinant insulin-like growth factor binding protein (Tressel *et al.*, 1991), recombinant interferon-gamma (IFN- γ , Curling *et al.*, 1989), recombinant t-PA (Moorhouse *et al.*, 1995) and recombinant Factor VIIa (Klausen and Kornfelt, 1995). The smaller rFC related peptide of 63 kDa may be due to the recognition of an internal methionine in the transcript as a start codon for

translation of the polypeptide. Such a feasible start site is M⁴⁶⁷ (Ding *et al.*, 1995), where the cDNA stretch ¹⁴¹³AATATGG¹⁴¹⁹ includes a perfect Kozak element.

COS-1 and *P. pastoris* derived rFCs are different in the pattern of their isoforms

Earlier studies on expression of this cDNA in the methylotrophic yeast *Pichia pastoris* (Roopashree *et al.*, 1996), have demonstrated that this yeast can produce significant levels of CrFC transcripts, as well as rFC protein of 130 kDa. On the other hand, the COS-1 derived rFC is comparable to that derived from insect cells, which also generated variants of the 130 kDa species (unpublished data). The present studies indicate that the production of rFC polypeptide differs significantly from lower to higher eukaryotic systems, in the size and number of rFC-related polypeptides. The differences in the patterns of rFC from yeast and COS-1 cells indicates the differences in its trafficking and post-translational modification in these two hosts.

A comparison between rFC and recombinant t-PA

Factor C, although a serine protease zymogen from a lower invertebrate organism, contains several features in its structural organisation (Muta *et al.*, 1991; Iwanaga, 1993), comparable to mammalian complement factors. In fact, it has been suggested that this similarity could be due to a common evolutionary origin for the two systems. This heterologous expression of CrFC could be compared to an extensively studied mammalian blood factor, the t-PA which is also a serine protease that bears a striking resemblance in its structural organisation to CrFC. Although recombinant t-PA from yeast is present in a single chain form (Lemmont *et al.*, 1985), when secreted by Bowes Melanoma cells, it is present in a mixture of glycosylated and unglycosylated forms of 68 and 65 kDa, as well as a cleaved double chain of 35 kDa. This pattern is similar to rFC polypeptides from COS-1 cells, suggesting that the 132 and 130 kDa are glycosylated and unglycosylated species, respectively. The 35 kDa t-PA species, analogous to the 63 kDa band of rFC, is believed to be the two chained form that occurred as a result of specific cleavage between the Arg-Ile sites, by its initiator plasmin present in the culture medium. In the amoebocytes of the horseshoe crabs, CrFC is present in both single and double chain forms (Ding *et al.*, 1993). Upon activation with LPS, the light chain of the latter species is further cleaved into A and B chains of 44 kDa and 8 kDa, respectively. This course of ligand-initiated activation of rFC or t-PA zymogens suggests another

possibility for the occurrence of the 63 kDa rFC band, via the cleavage of the 132 kDa rFC by COS-1 specific cell factors.

The rFC generated from COS-1 cells was associated with the cellular debris. In fact, membrane bound proteins are known to be expressed at much lower levels in COS-1 cells, which are capable of maintaining greater than 100,000 copies of the transfected DNA. Reduction in levels of protein is believed to be due to the lack of membrane surface area for which they can be deposited (Kaufman, 1990), which is perhaps the reason for low levels of rFC expression in COS-1 cells. These studies have however shown the feasibility of production of rFC in mammalian cell culture systems. Further studies on the membrane bound nature of rFC would be necessary to facilitate secretion of this complex molecule in heterologous systems.

Acknowledgements

We thank Mr. H.K. Yip for photography. Roopashree, S.D. is a recipient of Postgraduate Research Scholarship from the National University of Singapore. This work was supported by grant RP 95/0303 (JLD and BH).

References

- Buckler, A.J., Chang, D.D., Graw, S.L., Brook, D.J., Haber, D.A., Sharp, P.A. and Housman, D.E. (1991) *Proc. Natl. Acad. Sci.* 88, 4005–4009.
- Citarella, F., Aiuti, A., La-Porta, C., Russo, G., Peitropaolo, C. and Rinaldi, M. (1992) *Eur. J. Biochem.* 208, 23–30.
- Curling, E.M.A., Hayter, P.M., Baines, A.J., Bull, A.T., Gull, K., *et al.* (1989) *Biochem J.* 272, 333–337.
- Ding, J.L., Navas III, M.A.A. and Ho, B. (1993) *Biochim. et Biophys. Acta.* 1202, 149–156.
- Ding, J.L., Navas III, M.A.A., Ho, B. (1995) *Mol. Marine Bio. Biotech.* 4, 90–103.
- Gluzman, Y. (1981) *Cell* 23, 175–182.
- Ho, B. (1983) *Microbios Letters* 24, 81–84.
- Iwanaga, S. (1993) *Curr. Opinion Immunol.* 5, 74–82.
- Kaufman (1990) *Methods in Enzymol.* 185, 487–511.
- Klausen, N.K. and Kornfelt, T (1995) *J. Chromatogr.* 718, 195–202.
- Lemmont, J.F., Wei, C. and Dackowski, W.R. (1985) *DNA* 4, 419–428.
- Moorhouse, K.G., Eusebio, C.A., Hunt, G. and Chen, A.B. (1995) *J. Chromatogr.* 717, 61–69.
- Muta, T., Miyata, T., Misumi, Y., Tokunaga, F., Nakamura, J., Toh, Y., Ikehara, Y. and Iwanaga, S. (1991) *J. Biol. Chem.* 266, 6554–6551.
- Nakamura, T., Morita, T. and Iwanaga, S. (1986) *Eur. J. Biochem.* 154, 511–521.
- Philipova, D., Citarella, F., Venkov, P. and Fantoni, A. (1992) *Yeast* 8, Spec. Iss., S606.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, USA. edition.

- Ramanathan, L., Le, H.V., Labdon, J.E., Mays-Ichino, C.A., Syto, R. and Trotts, P.P. (1989) *Biochim. Biophys. Acta* 1007, 283–288.
- Roopashree, S.D., Chai, C., Ho, B. and Ding, J.L. (1995) *Biochem. Mol. Biol. Intl.* 35, 841–849.
- Roopashree, S.D., Ho, B. and Ding, J.L. (1996) *Mol. Mar. Biol. Biotech.* 5, 344–353.
- Tressel, T.J., Tatsume, G.P., Spratt, K. and Sommer, A. (1991) *Biochem. Biophys. Res. Commun.* 178, 625–633.

Received as Revised 25 February 1997

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q289014
Hikaru MIZUMURA, et al.	
Appln. No.: 18/346,420	Group Art Unit: 1656
Confirmation No.: 6959	Examiner: DAVID J STEADMAN
Filed: July 3, 2023	
For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN	

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated September 15, 2023, please amend the above-identified application as follows on the accompanying pages.

TABLE OF CONTENTS

AMENDMENTS TO THE CLAIMS	2
REMARKS	5

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (currently amended): A method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, ~~secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the~~[[a]] culture supernatant, wherein the horseshoe crab Factor C is a *Tachypleus tridentatus* Factor C or a *Limulus polyphemus* Factor C ~~comprising the horseshoe crab Factor C having activity of Factor C,~~

expressing the horseshoe crab Factor B ~~having activity of Factor B~~ in a mammalian cell as a host cell, ~~and recovering a culture supernatant comprising the horseshoe crab Factor B having activity of Factor B;~~

expressing the horseshoe crab Pro-clotting enzyme ~~having activity of Pro-clotting enzyme~~ in a mammalian cell as a host cell, ~~and~~

~~producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.~~

~~wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.~~

~~and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme; and~~

~~formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.~~

2. (original): The method according to claim 1, wherein the mammalian cell expressing the Factor B is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

3. (original): The method according to claim 1, wherein the mammalian cell expressing the Pro-clotting enzyme is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

4. (currently amended): The method according to claim 1, wherein the horseshoe crab ~~for Factor B and/or Pro-clotting enzyme~~ is selected from the group consisting of *Tachypleus tridentatus* ~~and~~ and *Limulus polyphemus*.

5. (original): The method according to claim 1, wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

6. (original): The method according to claim 1, wherein the horseshoe crab Factor C has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

7. (original): The method according to claim 1, further comprising filtering the culture supernatant comprising the horseshoe crab Factor C.

8. (original): The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are mixed in a formulation.

9. (original): The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are formulated separately.

10. (original): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing an endotoxin assay agent produced by the method according to claim 1 and a test specimen, and a step of measuring a progress of a cascade reaction.

REMARKS

In the present Amendment, claim 1 has been amended for clarification and to recite that the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different. Support for the amendment is found, for example, in paragraph [0075] of the present specification, which discloses that “In the endotoxin assay agent of the present invention, the factors and other components may be present as a mixture or present separately. For example, the factors may be mixed at any proportions in formulation, or individual factors may be formulated separately.” Claim 4 has been amended in view of the amendment to claim 1. No new matter has been added, and entry of the Amendment is respectfully requested.

Claims 1-10 are pending.

Response to Claim Objection

Claim 4 is objected to because, per the Examiner, the comma in the recitation of “Tachypleus tridentatus, and Limulus polyphemus” should be deleted.

In response, claim 4 has been amended to delete the comma. Accordingly, withdrawal of the claim objection is requested.

Response to § 112(b) and § 112(d) Rejections

Claim 9 is rejected under 35 U.S.C. § 112(b) as being indefinite. The Examiner states that the recitation of “wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are formulated separately” is inconsistent with the recitation

of “formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme” in claim 1.

Claim 8 is rejected under 35 U.S.C. § 112(d) as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. The Examiner states that given that claim 1 recites “formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme”, claim 8 does not further limit claim 1.

In response, claim 1 has been amended to delete the recitation “formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme”, and to recite that “the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different”.

In view of the amendment to claim 1, claim 8 further limits claim 1 and claim 9 is definite.

During a video interview with Primary Examiner Steadman conducted on November 14, 2023, the Examiner advised that the amendments presented herein would appear to overcome the current rejections.

In view of the above, reconsideration and withdrawal of the §112 rejections are respectfully requested.

Response to §103 Rejections

Claims 1-10 are rejected under 35 U.S.C. § 103 as being unpatentable over Ding et al. (US 5,858,706; cited on the IDS filed on July 3, 2023; hereafter “Ding-1”) in view of Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on July 3, 2023; hereafter “Tamura”).

Claims 1-10 are rejected under 35 U.S.C. § 103 as being unpatentable over Ding et al. (US 5,716,834; cited on the IDS filed on July 3, 2023; hereafter “Ding-2”) in view of Tamura.

The above two rejections should be withdrawn because Ding-1, Ding-2 and Tamura do not disclose or render obvious the claimed invention, either alone or in combination.

Claim 1 as amended relates to a method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the culture supernatant, wherein the horseshoe crab Factor C is a *Tachypleus tridentatus* Factor C or a *Limulus polyphemus* Factor C,

expressing the horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,

expressing the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme in a mammalian cell as a host cell, and

producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,

wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.

As the word "culture" indicates, "culture supernatant" refers to an extracellular fraction in culture process which is distinguished from cellular fraction. To further clarify the difference between "a supernatant by centrifuging cultured and lysed host cells" as described in Ding-1 and the claimed "culture supernatant", claim 1 has been amended to recite "secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the culture supernatant".

Ding-1 describes that "Recovery of rCrFC produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, Rather, intracellular rCrFC can be recovered by lysing the mammalian cells with detergents." (Example 1, Ding-1) That is, Ding-1 does **not** disclose "secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the culture supernatant" as defined in amended claim 1.

Regarding Ding-2, Ding-2 does **not** teach recovering a culture supernatant as recited in amended claim 1.

Before the priority date of the persent application, it was widely known to those skilled in the art that if mammalian cells are used as a host, Factor C is not secreted extracellularly but remains intracellularly. Specifically, the inventors (Jeak Ling Ding; Bow Ho) of Ding-1 and Ding-2 stated as follows after filing Ding-1 and Ding-2.

"The rFC generated from COS-1 cells was associated with the cellular debris." (page 360, right column, lines 4-5 of Roopashree et al, Biotechnology Letters Vol 19, No 4, 1997;

“rFC” is abbreviation of “recombinant Factor C”; Cos-1 cell is the mammalian cell exemplified in Ding-1 (column 9, line 41) and Ding-2 (column 20, line 29).)

“Earlier attempts in our lab to express rFC in E. coli, yeast and mammalian cells ... may not have met the requirements for Factor C folding and modification, thus rFCs expressed were sequestered inside these host cells, and only showed weak LPS-binding affinity.” (emphasis added.) (page 76, left column, lines 8-14 of Wang et al., Biotechnology Letters 23, 71-76, 2001)

On the other hand, contrary to the above common knowledge, the inventors of the present application have discovered that Factor C is secreted extracellularly when CHO cell or HEK cell are used among mammalian cells, as shown in the Examples of the present application.

Further, Applicant submits herewith a Declaration under 37 C.F.R. § 1.132 executed by Dr. Hikaru MIZUMURA, the first-named inventor of the above-identified application, presenting additional experimental data to show that while no Factor C activity was observed in the intracellular fraction prepared based on EXAMPLE 2 of Ding-1, excellent Factor C activity was observed in the culture supernatant as described in the Examples and Fig. 6 of the present application.

As shown in Figure 1, A and B, and Figure 2 at pages 3 and 4 of the Declaration, intracellular Factor Cs were confirmed to be present in CHO-K1, CHO DG44, CHO-S, and HEK cells transformed so as to express LpFC or TtFC.

In the Declaration, test samples for measuring Factor C activity of intracellular Factor C were prepared according to the procedure disclosed in EXAMPLE 2 of Ding-1. Factor C activity was measured in reference to a method disclosed in “4. Activity Measurement” section of the present specification.

The assay was conducted without endotoxin (blank) or under the presence of 0.1 EU/ml of endotoxin. Factor C activity was calculated as a difference of absorbance change rate between the measured value under the presence of 0.1 EU/ml of endotoxin and blank value. The culture supernatants of CHO, and HEK cells were used as positive controls. The results are summarized in Tables 1 and 2 at pages 5 and 6 of the Declaration (also shown below).

Table 1: LpFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μL)	Δ mAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.1
		4	0.0
	Culture supernatant	1	1.1
		2	16.1
		4	16.2
CHO-S	Cell lysate	1	-0.1
		2	0.1
		4	0.1
	Culture supernatant	1	2.5
		2	9.5
		4	12.9
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	11.2
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	7.3

Table 2: TtFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μL)	ΔmAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	0.3
		2	1.8
		4	6.4
CHO-S	Cell lysate	1	0.0
		2	-0.1
		4	0.0
	Culture supernatant	1	0.2
		2	8.5
		4	14.5
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	14.3
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	9.7

$\Delta mAbs/min = [mAbs/min \text{ at } 0.1 \text{ EU/ml}] - [mAbs/min \text{ at } 0 \text{ EU/ml}]$

[mAbs/min at 0.1 EU/ml]: Absorbance change rate at 0.1 EU/ml of Endotoxin concentration

[mAbs/min at 0 EU/ml]: Absorbance change rate without Endotoxin

Dr. Mizumura explains that as shown in Tables 1 and 2, Factor C activity of intracellular TtFC or LpFC was not detected in the samples prepared as in Ding-1.

Dr. Mizumura also explains that as seen from the above data, while no Factor C activity was observed in the intracellular fraction prepared based on EXAMPLE 2 of Ding-1, excellent Factor C activity was observed in the culture supernatant as described in the Examples and Fig. 6 of the present application. These results demonstrate superior effects provided by the claimed feature of "secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the culture

supernatant." The superior effects provided by the claimed invention are unexpected from Ding-1, Tamura et al. and Ding-2.

During a video interview with Primary Examiner Steadman conducted on November 14, 2023, the Examiner advised that the amendments, arguments, the additional data (presented in a Declaration under 37 C.F.R. § 1.132) and the supporting documents presented herein would appear to overcome the current rejections.

In view of the above, the present claims are not obvious and are patentable over Ding-1, Ding-2 and Tamura, either alone or in combination. Reconsideration and withdrawal of the §103 rejections based on Ding-1, Ding-2 and Tamura are respectfully requested.

Response to Double Patenting Rejections

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 8 of U.S. Patent No. 10,982,202 B2 (Cite No. 9 of the IDS filed on July 3, 2023; hereafter "patent") in view of Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1,3, 8, and 9 of U.S. Patent No. 10,144,923 B2 (Cite No. 2 of the IDS filed on July 3, 2023; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 4, and 10-12 of U.S. Patent No. 11,236,318 B2 (Cite No. 10 of the IDS filed on July 3, 2023; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

Claims 1 and 3-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,040,254 B2 (cited on the attached Form PTO-892; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 and 7-9 of U.S. Patent No. 11,499,177 B2 (cited on the attached Form PTO-892; hereafter “patent”) in view of Ding-1, Ding-2, and Tamura.

Claims 1-6, 8, and 9 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application).

Claims 7 and 10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application) in view of Tamura.

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3 of co-pending application 17/272,805 (reference application) in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1 and 7-9 of co-pending application 18/045,705 (reference application) in view of Ding-1, Ding-2, and Tamura.

Regarding the double patenting rejections based on U.S. Patent Nos. 10,982,202, 10,144,923, and 11,236,318, and co-pending application 17/554,128, Applicant submits herewith an E-Terminal Disclaimer to obviate the rejections.

Regarding the double patenting rejections based on U.S. Patent Nos. 9,040,254 and 11,499,177, and co-pending applications 17/272,805 and 18/045,705, the claimed invention utilizing culture supernatant of CHO or HEK cell shows unexpected superior effects over Ding-1, Ding-2, and Tamura, as discussed above with respect to the § 103 rejections.

In view of the above, reconsideration and withdrawal of all of the double patenting rejections based on the cited patents and reference applications are respectfully requested.

Allowance is respectfully requested. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: November 24, 2023

Attachments:

Roopashree et al, Biotechnology Letters Vol 19, No 4, 1997.

Wang et al., Biotechnology Letters 23, 71-76, 2001.

Declaration under 37 C.F.R. § 1.132 executed by Dr. Hikaru MIZUMURA.



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18/346,420

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Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY J oanna Sehy

PATENT CENTER # 63370356

FILING DATE 07/03/2023

CUSTOMER # 23373

FIRST NAMED INVENTOR Hikaru MIZUMURA

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Hui Wauters

Documents

TOTAL DOCUMENTS: 7

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Q289014_Executed Declaration 132.pdf	7	Affidavit-traversing rejections or objections rule 132	586 KB
Q289014_Amendment 111.pdf	14	-	92 KB
Q289014_Amendment 111- A....pdf	(1-1) 1	Amendment/Request for Reconsideration-After Non-Final Rejection	25 KB
Q289014_Amendment 111- CLM.pdf	(2-4) 3	Claims	40 KB
Q289014_Amendment 111-	(5-14) 10	Applicant Arguments/Remarks	75 KB

REM.pdf		Made in an Amendment	
Q289014_ 2001 Biotech Let_Wang.pdf	6	Applicant Arguments/Remarks Made in an Amendment	134 KB
Q289014_ 1997 Biotech Let_Roopashree.pdf	5	Applicant Arguments/Remarks Made in an Amendment	665 KB
Q289014_SSI.pdf	2	Applicant summary of interview with examiner	29 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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New International Application Filed with the USPTO as a Receiving Office

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Functional expression of full length *Limulus* Factor C in stably transformed Sf9 cells

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Abstract

Limulus Factor C is a potent antagonist of endotoxin from Gram-negative bacteria. A fusion construct containing full length Factor C has been cloned into *Spodoptera frugiperda* Sf9 cell. Stable Sf9 cell transfectants were obtained using Zeocin selection for 2 weeks. The recombinant Factor C (rFC) was secreted into the culture medium at 9 mg l⁻¹. Both the crude and partially purified rFC were able to detect lipid A at 10 pg ml⁻¹ in an ELISA-based lipid A binding assay.

Introduction

Factor C, a serine protease zymogen from the amoebocytes of the horseshoe crab, is sensitive to endotoxin, or lipopolysaccharide (LPS) (Nakamura *et al.* 1986). It initiates the coagulation cascade in the horseshoe crab haemolymph (Nakamura *et al.* 1986) and thus plays a vital role in the defence against invading Gram-negative bacteria. Owing to the high affinity of Factor C for endotoxin (Ding *et al.* 1997, Tan *et al.* 2000), LPS can be detected at fg ml⁻¹ in a modified *Limulus* amoebocyte lysate (LAL) gelation assay (Ho *et al.* 1983). The LAL assay is used widely in the quality assurance of biological and pharmaceutical preparations. However, due to over-harvesting of horseshoe crab for amoebocyte lysate preparations, the species is now endangered. It is therefore imperative to obtain a second generation of 'Limulus amoebocyte lysate' (LAL) by recombinant DNA technology.

Expression of recombinant Factor C, rFC, in heterologous hosts have been carried out using various systems: *E. coli* (Roopashree *et al.* 1995), *Pichia pastoris* (Ding *et al.* 1996), *Saccharomyces cerevisiae* (Roopashree *et al.* 1997b), and COS-1 (Roopashree *et al.* 1997a). However, none of these recombinant proteins were secreted and they remained insoluble

within the host cells, indicating that the native signal sequence was not able to direct full length rFC out of these heterologous hosts.

In the current study, a new approach for production and secretion of rFC is carried out. Our laboratory has recently developed a strong novel secretory signal, ss (US Patent filed). By replacing the native signal peptide of CrFC21 with ss, we are able to obtain soluble rFC in the culture supernatant. *Spodoptera frugiperda* (Sf9) cell was chosen as the expression host, since it is genetically closer to arthropod than any other heterologous hosts used in earlier experiments. We describe here the expression pattern of the full-length Factor C in stably transformed Sf9 cells, and the functionality of rFC is also investigated.

Materials and methods

All restriction and DNA modifying enzymes were purchased from Boehringer Mannheim and New England Biolabs.

Construction of expression plasmid

Lepidopteran insect expression vector, pIZT/V5-His (Invitrogen) was used for fusion expression. The na-

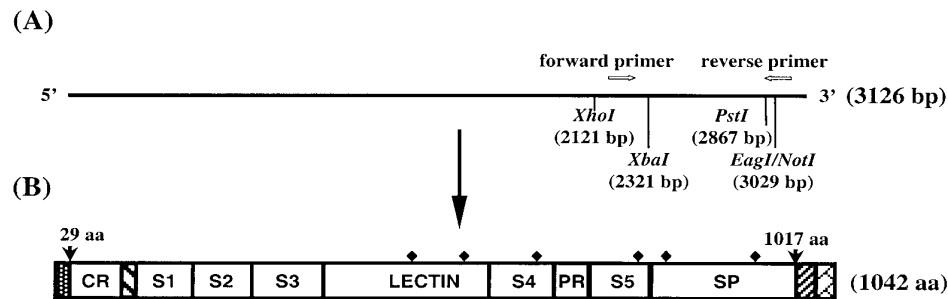


Fig. 1. (A) Recombinant Factor C cDNA. Primers used for PCR are presented by open arrows ($\rightleftharpoons$). Restriction sites used for expression vector construction and Southern blot probe preparation are also indicated. (B) Domain structure of the recombinant Factor C. The fusion sites at *N*- and *C*-terminus are indicated by closed arrows ($\downarrow$) with the amino acids numbered corresponding to the original CrFC21 (GenBank Accession No: S77063). The potential glycosylation sites are marked with diamonds ($\blacklozenge$). $\blacksquare$: Secretory signal (ss); CR: cysteine-rich region; $\boxminus$: epidermal growth factor-like domain; S1–5: sushi-like domains 1–5; LECTIN: lectin-like domain; PR: proline-rich region; SP: serine-protease coding region; $\boxplus$: V5 epitope; $\boxtimes$: 6 $\times$ His tag.

tive signal peptide sequence of full length cDNA, CrFC21 (Ding *et al.* 1995), was replaced by a novel secretion signal, ss, to direct the fusion protein out of the host cells. The cDNA fragment containing the entire open reading frame was obtained by digestion with *KpnI* and *NotI* from pBluescript II SK vector (Stratagene), and inserted into compatible sites in pIZT/V5-His. A pair of primers (forward: 5'-GATGGATCCTCGTTCCTTTATC-3'; reverse: 5'-ATCGGCCGCCATAATCCATGACAGG-3') with *BamHI* and *EagI* sites (underlined) were used to obtain the 786 bp 3'-terminal portion of Factor C cDNA (Figure 1A). PCR was performed with a hot start at 94 °C for 2 min, followed by 30 cycles of two-min denaturation at 94 °C, 1 min of annealing at 60 °C, and 1 min of extension at 72 °C. This fragment was subsequently digested with *XbaI* and *EagI* to replace the *XbaI*-*NotI* fragment in native Factor C cDNA, allowing the removal of stop codon as well as in-frame fusion between the cDNA and V5-epitope/6 $\times$ His tag in the expression vector. Figure 1B depicts the domain structures of the recombinant construct. In pIZT/ssFLFC-V5-His, 29-1017 aa of CrFC21 (Ding *et al.* 1995) was fused between ss at the *N*-terminus end and V5-epitope/6 $\times$ His tag at the *C*-terminus. In this construct, the recombinant full length Factor C cDNA was placed under transcriptional control of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus immediate-early 2 gene promoter, OpIE2 (Theilmann & Steward 1992). In addition, it contains a Zeocin resistance gene for selecting transformants in Zeocin-containing growth medium. The construct

was transformed into competent *E. coli* TOP10F', and the DNA sequence was verified by BigDye Terminator Cycle Sequencing system (Perkin-Elmer, ABI Prism377).

In vitro coupled transcription-translation (TnT)

To ensure the expression competency of the construct, the cDNA insert was digested by *KpnI* and *EagI*, and recloned into compatible sites in pBluescript II SK vector containing T7 promoter. The resultant recombinant construct, pBS/ssFLFC-V5-His, was subjected to TnT reaction (Promega) in standard volumes of 50 μ l each containing 2 μ g of plasmid DNA. [^{3} H]-Leucine (Amersham, UK) was used to monitor the efficiency of the reaction which was carried out at 30 °C for 90 min. The TnT products were resolved by 10% (w/v) SDS-PAGE and the gel was stained with Coomassie Blue for 30 min, fluorographed for 30 min with Amplify (Amersham), dried and autoradiographed for 16 h at -70 °C. Autoradiograms were developed using a Kodak X-Omat processor.

Cell culture and transfection

Sf9 insect cells were maintained in Sf-900 II SFM (Gibco) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Clontech), 50 U penicillin ml^{-1} and 50 μ g streptomycin ml^{-1} . The cells were incubated as monolayers at 27 °C in a humidified incubator. Two ml cells, 1×10^6 cells ml^{-1} , in 35 mm wells (Iwaki) were transfected with 3.3 μ g CsCl-purified pIZT/ssFLFC-V5-His DNA mediated

by 6.7 μ l Insectin-Plus (Invitrogen) in 200 μ l Sf-900 II SFM without supplement and antibiotics. After transfection, cells were maintained in the DNA-lipid complex for 5 h. Subsequently, the solution was removed and replaced by 2 ml fresh medium. Cells were allowed to recover for 2 days before selection. Stable transfectants were selected with Zeocin (Invitrogen) at 1000 μ g ml⁻¹ for 2 weeks. Stable cell lines expressing rFC were adapted to Sf-900 II SFM by weaning over four to five passages. Subsequently, Sf9 transfectants were cultured in suspension in a 250-ml spinner flask (Bellco, USA) containing 100 ml Sf-900 II SFM, and further scaled up to a 500-ml Bellco spinner flask containing 200 ml medium. Cell cultures were harvested at mid-growth phase at $3\text{--}4 \times 10^6$ cell ml⁻¹. All procedures were carried out under pyrogen-free conditions.

Genomic DNA analyses

Total genomic DNA was isolated from Sf9 stable transfectants using InstaGene Matrix (BioRad) according to the manufacturer's instructions. To confirm the integration of the pIZT/ssFLFC-V5-His in the Sf9 cell genome, the region corresponding to the 3' terminal portion of CrFC21 cDNA was amplified using specific forward and reverse primers as described. The PCR-derived product was used for Southern blotting. An *Xho*I-*Pst*I fragment located at the 3'-terminus of CrFC cDNA was [³²P] labelled and used as the specific probe (Figure 1A).

Western analyses of intracellular and secreted rFC proteins

The three protein fractions of interest are defined as: (1) M: protein secreted into medium, which was obtained by clarification of conditioned medium at 14000 $\times$ g for 5 min at 4 °C; (2) L: soluble cell lysate derived from cells of 2 ml culture that was lysed in 100 μ l PBS by 5 cycles of freeze-thawing at -70 °C and 37 °C, followed by centrifugation at 14000 g for 15 min at 4 °C; and (3) P: the resultant insoluble fraction containing particulate proteins. Fraction P was solubilised in 100 μ l SDS-PAGE sample buffer containing 50 mM Tris/HCl, pH 6.8, 2% (w/v) SDS and 2% (v/v) β -mercaptoethanol. The total soluble protein in medium (M) and cell lysate (L) was measured using Bradford method. Western analyses of the rFC proteins using anti-V5-HRP antibody (Invitrogen) was carried out. The blots were visualised using the Supersignal West Pico Chemiluminescent

Substrate (Pierce). The relative amounts of rFC in the three fractions were quantitated densitometrically by using ImageMaster (Pharmacia) software. Recombinant Factor C production was estimated in comparison to a purified protein containing V5 epitope in Western blot analysis.

Concentration and partial purification of secreted rFC samples

Culture supernatant was collected after centrifugation of cell culture at 3000 $\times$ g for 10 min at 4 °C to remove all the cells. The supernatant was concentrated 10 times by ultrafiltration using regenerated cellulose membrane cassette (Pellicon XL, 30 kDa molecular weight cut-off, MWCO, Millipore). Partial purification was carried out by gel filtration chromatography through Sephadex G-100 (1.5 $\times$ 90 cm), using 0.05 M Tris/HCl (pH 7.5), 0.154 M NaCl as running buffer. Fractions of 1 ml were collected, and the fractions corresponding to the void peak were pooled and concentrated using Centriprep regenerated cellulose membrane (30 kDa MWCO, Amicon YM).

Endotoxin-binding analysis

The LPS-binding potential of the secreted soluble rFC protein was analysed by an ELISA-based assay for lipid A, the biologically active component of LPS.

One hundred μ l of diphosphoryl lipid A from *E. coli* F-583 (Sigma) of different concentrations were coated on the wells of a polysorp 96-well plate (Nunc) by incubating at 37 °C for 2 h. The wells were washed 3 times with 200 μ l of wash solution [pyrogen-free PBS containing 0.01% (v/v) Tween-20, 0.01% (w/v) merthiolate], and blocked overnight at room temperature with pyrogen-free PBS containing 0.2% (w/v) BSA, and 0.01% (v/v) Tween-20. After 3 washes, 100 μ l samples containing 10 μ g protein was added into each well, followed by 16 h of incubation at room temperature. The samples were removed and the wells were washed 3 times. Subsequently, 100 μ l rabbit anti-Factor C antibody (1:500 dilution) was added and incubated for 3 h at 37 °C followed by 3 washes before addition of 100 μ l goat anti-rabbit secondary antibody (1:2000 dilution) conjugated to horseradish peroxidase (Dako). Finally, 100 μ l ABTS solution (Boehringer Mannheim) was used as substrate. The absorbance of the samples was determined at 405 nm with reference wavelength at 490 nm using the SPECTRAmax 340 plate reader, with SOFTmax PRO version 1.2.0.

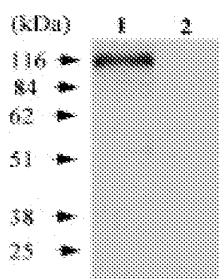


Figure 2. Autoradiogram of TnT product from pBS/ssFLFC-V5-His expression construct. Ten μ l of the total reaction mix (50 μ l) was loaded onto each lane of 10% (w/v) SDS polyacrylamide gel under reducing conditions. ^{3} [H]-Leucine labelled TnT products from pBS/ssFLFC-V5-His (lane 1) and pBluescript II SK vector DNA as negative control (lane 2).

Results

Construction of pIZT/ssFLFC-V5-His and verification of expression competency

After removal of the native signal sequence and stop codon, the CrFC cDNA was inserted between a novel secretory signal sequence and 3'-end V5 epitope and 6 $\times$ His tag coding sequence, resulting in pIZT/ssFLFC-V5-His. This recombinant plasmid, therefore, contains the native CrFC sequence encoding amino acid, 29-1017. To confirm the efficacy of the start codon recognition, and to rule out the existence of any premature stop codon introduced during the cloning process, TnT (*in vitro* coupled transcription translation) analyses were carried out. The cDNA insert from pIZT/ssFLFC-V5-His was re-cloned into the T7 promoter containing vectors which allowed TnT experiments. The expression construct, pBS/ssFLFC-V5-His was thus obtained. The presence and orientation of the inserts were verified by restriction analysis and sequencing. The 3126 bp cDNA insert generated a 116 kDa polypeptide corresponding to its calculated molecular mass (Figure 2). The absence of other minor bands indicates strong and specific recognition of start codon and correct termination of translation in the reticulocyte lysate system. The integrity of the original cDNA in pIZT/V5-HisA was thus verified and used for *in vivo* expression studies in Sf9 cells.

Genomic DNA analysis

After two weeks of selection, total DNA from the stable Sf9 cell transfectants was extracted and used as template for PCR to test for the integration of the

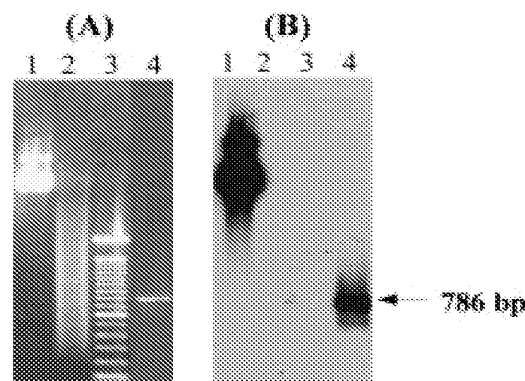


Figure 3. PCR and Southern blot analysis of integration of Factor C cDNA into Sf9 cell genome. (A) Electrophoresis of PCR product in 1% (w/v) agarose gel. Lane 1: positive control: pIZT/ssFLFC-V5-His plasmid DNA; lane 2: negative control: PCR product from wild type Sf9 cell genomic DNA; lane 3: molecular weight standards: 100 bp ladder DNA marker (Gibco BRL); lane 4: PCR product from stable Sf9 transformant genomic DNA. Primers used for PCR are indicated in Figure 1. (B) Southern hybridization of PCR fragments. The gel was Southern blotted and probed with ^{32} P labelled *XhoI-PstI* fragment (the 3' end of CrFC21; see Figure 1A)

expression vector. The PCR product of 786 bp was generated only from the transfected Sf9 cell DNA (Figure 3A). Southern analysis of this product showed this fragment hybridized strongly and specifically with the homologous ^{32} P CrFC21/*XhoI-PstI* probe (Figure 3B). It indicates that the rFC gene was indeed intact and the expression cassette was inserted into the Sf9 cell genome.

Transfected Sf9 cells expressed full length rFC

By using anti-V5-HRP antibody as probe, rFC was detected in transient transfection studies as early as 24 h post-transfection (data not shown). This indicates a successful readthrough of the CrFC sequence into the V5-His fusion tag. The immunoreactive rFC was 132 kDa in size (Figure 4), compared to the 116 kDa band observed by TnT. It indicates that extensive post-translational modifications were carried out in the host Sf9 cell. Furthermore, the intracellular rFC was observed in both soluble and insoluble fractions. These intracellular rFCs had lower mobility in the blot than the extracellular rFC, which indicates the cleavage of the signal peptide and glycan modification during the secretory pathway. The majority of rFC protein was secreted into the culture medium. According to densitometric scan, 84% of the total rFC protein was present

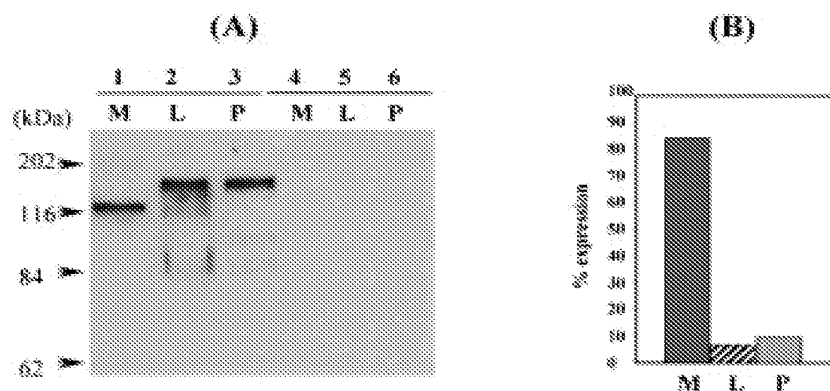


Figure 4. (A) Western blot analysis of protein samples from M (culture medium, 1.0 μg), L (soluble cell lysate, 10 μg) and P (insoluble fraction, 10 μg). Lanes 1, 2, 3: protein samples from Sf9 stable transfectant; lanes 4, 5, 6: protein samples from wild type Sf9 cells. Sizes of molecular markers are indicated at the left. (B) Percentage of rFC present in three fractions, M (84%), L (6%) and P (10%). Since M contains 84% of expressed rFC, only 1.0 μg of secreted proteins was used in the Western blot to assure the exposure was within the linear range of film and scanner response.

in the culture supernatant, while only 6% and 10% of the recombinant protein were localized in the soluble cell lysate (L) and insoluble particulate fraction (P), respectively. By comparison with a purified protein (>99% purity) containing V5-epitope in Western blot, the expression level of rFC in culture supernatant was determined to be 9 mg l^{-1} .

The His-antibody unexpectedly failed to detect recombinant protein in immunoblotting. The cleavage of His-tag is probably due to proteolysis of the carboxyl end of the recombinant proteins, by intra- or extra-cellular proteases.

Secreted rFC is functional

The rFC from Sf9 cells was tested for their functionality using ELISA (Figure 5). A broad range of concentration of lipid A was used in the assay. In lipid A-based ELISA, rFC protein showed apparent binding compared to the control medium. As low as 10 pg ml^{-1} lipid A was detected in the assay. As the concentration of lipid A was increased, we consistently obtained increasing OD readings of the ABTS substrate. Gel filtration enriched the rFC proteins, resulting in 33% increase in binding, indicating higher sensitivity to lipid A after removal of the small size molecules and ions, which may interfere with the binding.

Although intracellular rFC was also obtained in this system, it only accounts for a small percentage of total rFC expressed, and due to numerous endoge-

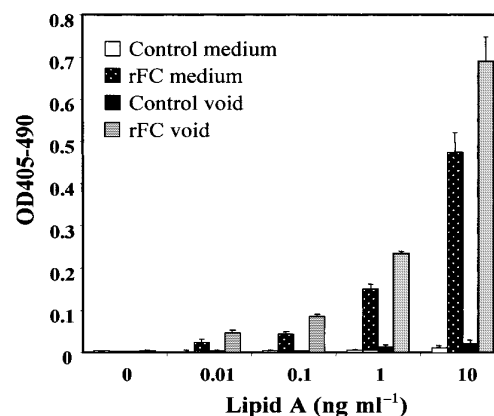


Figure 5. ELISA-based lipid A binding assay of rFC samples from Sf9 cells. Ten μg protein samples were used in the assay. Control medium: concentrated serum free medium from untransfected Sf9 cells; rFC medium: concentrated serum free medium from stable Sf9 cell line expressing rFC; control void: protein sample concentrated from the void fraction of control medium passed through Sephadex G-100 column; rFC void: protein sample concentrated from the void fraction of rFC medium passed through Sephadex G-100 column.

nous proteins, additional steps may be necessary to yield biologically active rFC, and thus limits its application value. Thus the 84% secreted and functional rFC offers an advantageous alternative to LAL.

Discussion

Factor C is a large complex multi-domain protein of 132 kDa, and has six potential *N*-glycosylation sites. As delineated by earlier studies, the *N*-terminus of Factor C contains intricately folded cysteine-rich and EGF-like domains followed by the important LPS-binding domain. Further downstream, there is a proline-rich region which shares homology with the hinge region of human coagulation Factor XII. Earlier attempts in our lab to express rFC in *E. coli*, yeast and mammalian cells (Roopashree *et al.* 1995, 1996, 1997, Ding *et al.* 1997) may not have met the requirements for Factor C folding and modification, thus rFCs expressed were sequestered inside these host cells, and only showed weak LPS-binding affinity (Ding *et al.* 1997, Pui *et al.* 1997, 1998).

In this work, we have used Sf9 cell as a host for expression of rFC, and a new approach was employed to create stable cell lines that continuously produce the recombinant protein of interest. This method differs from the conventional baculovirus expression system in that non-lytic, long-term stable expression is allowed without viral infection at every cycle of expression, so that significant time and effort is saved. A stable polyclonal population of Sf9 transformant was obtained in only two weeks. In our case, the expression level of rFC remained constant over 30 passages without Zeocin selection.

By introducing the novel secretory signal (ss) into Factor C, we successfully detected the protein in the culture supernatant. The expression level we observed is relatively high (9 mg l⁻¹). It is comparable to other recombinant proteins expressed under the OpIE2 promoter (Hegedus 1999). Fusion of C-terminal epitope allows easy detection of the rFC. The unexpected failure of detecting rFC utilizing 6 × His tag Probe indicates the trimming of the full length rFC, suggesting employment of alternative tag for detection and purification of the rFC, for example, 10 × His tag.

As shown by ELISA, the soluble and secreted form of rFC is functional in specifically recognising and binding lipid A. Sf9 cell itself secretes only low level of endogenous protein, thus reducing the steps required for its purification. The assay shows that rFC is of practical value in detecting trace amounts of LPS.

In conclusion, we have utilised a new approach for the production of rFC. The secreted Factor C conforms to the expected size, is immunoreactive and biologically active. This system offers advantages for the production of rFC, due to its high expression, ca-

pability of scale-up culture in bioreactors, efficient secretion and ease of purification. This recombinant Factor C may substitute conventional 'LAL' assay for mass detection of LPS in the near future.

Acknowledgements

This work was supported by grants from NUS (RP970319) and NSTB (LS/99/004). J. Wang is a postgraduate research scholar of the National University of Singapore.

References

- Ding JL, Chai C, Pui WM, Ho B (1997) Expression of full length and deletion homologues of *Carcinoscorpius rotundicauda* Factor C in *Saccharomyces cerevisiae*: immunoreactivity and endotoxin binding. *J. Endotoxin Res.* 4: 33–43.
- Ding JL, Navas MAIII, Ho B (1995) Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*. *Mol. Mar. Biol. Biotech.* 4: 90–103.
- Ding JL, Tan NS, Ho B, Lam TJ (1996) Isolated nucleic acids encoding a secretory signal for expression and secretion of heterologous recombinant proteins. US Patent Application No. 09/426, 776; PCT/SG99/00108, WO 00/26366.
- Hegedus DD, Pfeifer TA, Theilmann DA, Kennard ML, Gabathuler R, Jefferies WA, Grigliatti TA (1999) Differences in the expression and localization of human melanotransferrin in lepidopteran and dipteran insect cell lines. *Protein Express. Purif.* 15: 296–307.
- Ho B (1983) An improved *Limulus* gelation assay. *Microbios. Lett.* 24: 81–84.
- Nakamura T, Morita T, Iwanaga S (1986) Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in *Limulus* hemocytes. Isolation and characterization. *Eur. J. Biochem.* 154: 511–521.
- Pui AWM, Ho B, Ding JL (1997) Yeast recombinant Factor C from horseshoe crab binds endotoxin and causes bacteriostasis. *J. Endotoxin Res.* 4: 391–400.
- Pui AWM, Roopashree SD, Ho B, Ding JL (1998) Recombinant Factor C from *Carcinoscorpius rotundicauda* binds endotoxin. In: *New Development in Marine Biotechnology*. New York: Plenum Press, pp. 151–154.
- Roopashree SD, Chai C, Ho B, Ding JL (1995) Expression of *Carcinoscorpius rotundicauda* Factor C cDNA. *Biochem. Mol. Biol. Intl.* 35: 841–849.
- Roopashree SD, Ho B, Ding JL (1996) Expression of *Carcinoscorpius rotundicauda* Factor C in *Pichia pastoris*. *Mol. Mar. Biol. Biotech.* 5: 334–343.
- Roopashree SD, Ho B., Ding JL (1997a) Recombinant COS-1 cells express *Carcinoscorpius rotundicauda* Factor C. *Biotechnol. Lett.* 19: 357–361.
- Roopashree SD, Ho B, Ding JL (1997b) The cys-rich and EGF-like domains of *Carcinoscorpius rotundicauda* Factor C yields soluble fusion protein with GFP. *Biotechnol. Lett.* 19: 1147–1150.
- Tan NS, Ho B, Ding JL (2000) High-affinity LPS binding domain(s) in recombinant Factor C of a horseshoe crab neutralizes LPS-induced lethality. *FASEB J.* 14: 859–870.
- Theilmann DA, Steward S (1992) Molecular analysis of the transactivating IE-2 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* 187: 84–96.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q289014

Hikaru MIZUMURA, et al.

Appln. No.: 18/346,420

Group Art Unit: 1656

Confirmation No.: 6959

Examiner: DAVID J STEADMAN

Filed: July 3, 2023

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

STATEMENT OF SUBSTANCE OF INTERVIEW

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Please review and enter the following remarks summarizing the interview conducted on
November 14, 2023:

REMARKS

An Examiner's Interview Summary Record (PTO-413) was dated November 17, 2023.

As an initial matter, Applicant's representative would like to thank Primary Examiner
Steadman for the courtesies extended during the video interview conducted November 14, 2023.

The Interview Summary mailed November 17, 2023 provides an accurate summary and
statement of the substance of the interview.

It is respectfully submitted that the instant STATEMENT OF SUBSTANCE OF
INTERVIEW complies with the requirements of 37 C.F.R. §§1.2 and 1.133 and MPEP §713.04.

It is believed that no petition or fee is required. However, if the USPTO deems
otherwise, Applicant hereby petitions for any extension of time which may be required to

STATEMENT OF SUBSTANCE OF INTERVIEW
Appln. No.: 18/346,420

Attorney Docket No.: Q289014

maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

/Hui C. Wauters/

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

Hui C. Wauters
Registration No. 57,426

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: November 24, 2023



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PATENT AND TRADEMARK OFFICE**

Commissioner for Patents
United States Patent and Trademark Office
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Alexandria, VA 22313 - 1450
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APPROVAL LETTER

APPLICATION #
18/346,420

FILING DATE
07/03/2023

APPLICANT/PATENT UNDER REEXAMINATION
Hikaru MIZUMURA

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD
FOR MEASURING ENDOTOXIN

Electronic terminal disclaimer filed on 11/24/2023

☒ Approved

This patent is subject to a Terminal Disclaimer

Approved / Disapproved by: Electronic Terminal Disclaimer automatically approved



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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
11/24/2023 12:23:06 PM Z ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Hui Wauters

PATENT CENTER # 63370103

FILING DATE 07/03/2023

CUSTOMER # 23373

FIRST NAMED INVENTOR Hikaru MIZUMURA

CORRESPONDENCE ADDRESS -

AUTHORIZED BY -

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
petition-request.pdf	3	Terminal Disclaimer-Filed (Electronic)	50 KB
grantLetter.pdf	1	Terminal Disclaimer-Electronic- Approved	19 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
petition-request.pdf	B9A3E617036C85C75BFD8F77B267C543E64B46AD5CDEE9A6 22F19DF23FBA32BDF23AFB01B6B94F9805662D25E494C744D

588BB118367F48F3D098F551B38D317

grantLetter.pdf

EF9D511781B4AB164542B7F8E716A2A4DC30F87A950100452A
02BDAEDC17CD4561D0065B19A1FC8FA2582F5A295879D36D2
091162633883D39B22B5B0DC86B8B

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



ELECTRONIC PAYMENT RECEIPT

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18/346,420

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11/24/2023 12:23:06 PM Z ET

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Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Hui Wauters

PATENT CENTER # 63370103

AUTHORIZED BY -

CUSTOMER # 23373

FILING DATE 07/03/2023

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Hikaru MIZUMURA

Payment Information

PAYMENT METHOD
DA / 194880

PAYMENT TRANSACTION ID
E2023ANC24298293

PAYMENT AUTHORIZED BY
Hui Wauters

PRE-AUTHORIZED ACCOUNT
194880

PRE-AUTHORIZED CATEGORY
37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees); 37 CFR 1.19 (Document supply fees); 37 CFR 1.21 (Miscellaneous fees and charges)

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1814	STATUTORY DISCLAIMER, INCLUDING TERMINAL DISCLAIMER	170.00	1	170.00
TOTAL AMOUNT:				\$170.00

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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION

APPLICATION #
18346420

FILING DATE
07/03/2023

FIRST NAMED INVENTOR
Hikaru MIZUMURA

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD
FOR MEASURING ENDOTOXIN



Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action



This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent interest
SEIKAGAKU CORPORATION	100%
Total	100%

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

Application #	Filing Date
17554128	12/17/2021

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so

granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

Patent #
10982202
10144923
11236318

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:

- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
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Terminal disclaimer fee under 37 CFR 1.20(d) included with Electronic Terminal Disclaimer request.



Applicant claims the following entity status:

Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I certify, in accordance with 37 CFR 1.4(d)(4) that I am: An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

Signature	Name	Registration #
/Hui C. Wauters/	Hui Wauters	57426

* Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this certification. See MPEP 324.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 18/346,420		Filing Date 07/03/2023		☐ To be Mailed			
ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO											
APPLICATION AS FILED - PART I											
		(Column 1)	(Column 2)								
FOR		NUMBER FILED	NUMBER EXTRA	RATE (\$)		FEE (\$)					
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A	N/A	N/A							
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A	N/A	N/A							
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A	N/A	N/A							
TOTAL CLAIMS (37 CFR 1.16(j))		minus 20 =	*	x \$100 =							
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =	*	x \$480 =							
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).									
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))											
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL							
APPLICATION AS AMENDED - PART II											
		(Column 1)	(Column 2)	(Column 3)							
AMENDMENT	11/24/2023	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)				
	Total (37 CFR 1.16(i))	* 11	Minus ** 20	= 0	x \$100 =		0				
	Independent (37 CFR 1.16(h))	* 1	Minus *** 3	= 0	x \$480 =		0				
	☐ Application Size Fee (37 CFR 1.16(s))										
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))										
					TOTAL ADD'L FEE		0				
AMENDMENT		(Column 1)	(Column 2)	(Column 3)							
		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)				
	Total (37 CFR 1.16(i))	*	Minus **	=	x \$0 =						
	Independent (37 CFR 1.16(h))	*	Minus ***	=	x \$0 =						
	☐ Application Size Fee (37 CFR 1.16(s))										
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))											
					TOTAL ADD'L FEE						
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.					LIE						
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".					/CHRISTINE V MOORE/						
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".											
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.											

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959
23373	7590	11/17/2023	EXAMINER	
SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 9000 WASHINGTON, DC 20006			STEADMAN, DAVID J	
			ART UNIT	PAPER NUMBER
			1656	
			NOTIFICATION DATE	DELIVERY MODE
			11/17/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PPROCESSING@SUGHRUE.COM
USPTO@sughrue.com
sughrue@sughrue.com

<i>Applicant-Initiated Interview Summary</i>	Application No. 18/346,420	Applicant(s) MIZUMURA et al.		
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA (First Inventor to File) Status Yes	Page 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	Title	Type
DAVID J STEADMAN	Primary Examiner	Video Conference
HUI C WAUTERS	Attorney of Record	

Date of Interview: 14 November 2023

Issues Discussed:

Proposed Amendment(s)

An interview agenda including a proposed amendment (Appendix A), experimental data (Appendix B), and references (Appendices C and D) were presented to the examiner. The examiner presented a revision to the applicant's proposed claim 1 (Appendix E) for discussion. The examiner noted that the revised claim 1 explicitly recited secreting the Factor C into the culture medium and recited the particular horseshoe crabs for the Factor C, which are consistent with the applicant's argument and experimental data. The examiner noted that the revised claim 1 recited the step of "producing an endotoxin assay agent..." to complete the method. No agreement was reached as to the patentability of the claims.

☒ Attachment

/David Steadman/ Primary Examiner, Art Unit 1656	
<p>Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04</p> <p>Please further see: MPEP 713.04 Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b) 37 CFR § 1.2 Business to be transacted in writing</p>	

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Recombinant COS-1 cells express *Carcinoscorpius rotundicauda* Factor C

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The cDNA encoding *Carcinoscorpius rotundicauda* Factor C, CrFC, when expressed in mammalian cells, produced full length and truncated variants of rFC. In an *in vitro* coupled transcription-translation system, aberrant products were generated from pCDNA1/CrFC21, as opposed to pCIneo/CrFC21 which gave rise to the expected polypeptide. COS-1 cells transfected with pCIneo/CrFC21 expressed Factor C-related proteins which differ from those produced in the yeast, *Pichia pastoris*. The expression of a complex, mosaic protein such as the rFC in mammalian cells also resembled other mammalian blood factors, indicating that such cells could be used for production of a second generation 'limulus lysate'.

Introduction

The defense mechanism of the horseshoe crab against invading pathogens consists of an amoebocyte-mediated coagulation cascade. Trace amounts of lipopolysaccharide (LPS) from the Gram-negative bacterial cell wall activates the key enzyme, Factor C (FC), which initiates the cascade to result in a gel clot (Nakamura *et al.*, 1986). Extremely sensitive gelation (Ho, 1983) and colorimetric assays (Miura *et al.*, 1992) are available for the detection of activated FC. This forms the basis for the Limulus amoebocyte lysate (LAL) test that is widely used in pharmaceutical industry for endotoxin detection. However, overharvesting of the horseshoe crabs has led to their near-extinction in recent years. Towards obtaining a second generation 'limulus lysate', Factor C from the Southeast Asian horseshoe crab, *Carcinoscorpius rotundicauda* (CrFC), has been studied extensively. A single chain form of 132 kDa, as well as a double chain form of 80 and 52 kDa have been purified to homogeneity (Ding *et al.*, 1993). Two full length cDNAs, CrFC26 of 4182 bp and CrFC21 of 3448 bp have been cloned and characterised (Ding *et al.*, 1995). These two cDNAs differ mainly in the length and nucleotide composition of their 5' and 3' untranslated regions and have a 99.1% homology in the coding region. The CrFC26 and 21 as well as their truncated forms have been studied in *in vitro* coupled transcription-translation (TnT) assays using rabbit reticulocyte lysate (Roopashree *et al.*, 1995). These assays generated the expected translated products with full-length CrFC21 constructs, thus forming the basis of our choice of this cDNA species for expression in heterologous systems.

Being endotoxin-free, the methylotrophic yeast, *Pichia pastoris* was used to produce high amounts of CrFC transcripts, as well as recombinant Factor C (rFC) that was possibly membrane bound (Roopashree *et al.*, 1996).

Other comparable serine proteases such as Factors V, VII, IX, X, and XII (Citarella *et al.*, 1992), and the human tissue type plasminogen activator (t-PA), with structural organisations similar to CrFC have been expressed in mammalian cells. Expression of complex proteins in lower eukaryotic yeast systems may differ from higher eukaryotes, as reported in the case of both Factor XII and t-PA (Citarella *et al.*, 1992; Philipova *et al.*, 1992 and Lemmont *et al.*, 1985, respectively). Accordingly, expression of CrFC in mammalian cells such as COS-1 would offer the advantages of all post-translational modifications that could be offered in a heterologous system. It would be of immense interest to study the expression of an invertebrate 'complement like' factor in mammalian cells, such as COS-1. These studies would also determine the feasibility of establishing a permanent cell line that can produce rFC.

Materials and methods

Construction of recombinant expression vectors (Figs 1 and 2)

All cloning procedures were carried out according to Sambrook *et al.* (1989). Unless co-transfected with a second plasmid encoding a gene for drug resistance, the vector pCDNA1 is only designed for transient expression. On the other hand, cells transfected with pCIneo

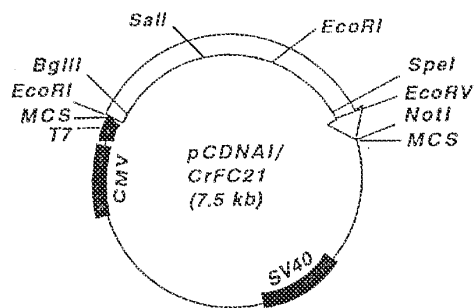


Figure 1 Recombinant construct pCDNAI/CrFC21. The vector pCDNAI (Invitrogen, USA) was first digested with EcoRI and NotI, to receive the 1.1 kb fragment of CrFC21 flanked by EcoRI and NotI (Roopashree et al., 1995), generating the intermediate construct pCDNAI/CrFC21EN. This plasmid was then linearised with EcoRI and ligated with the remaining 2.316 kb EcoRI flanking fragment of CrFC21, to create the complete recombinant vector pCDNAI/CrFC21.

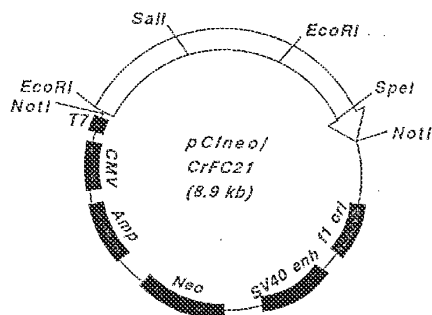


Figure 2 Recombinant construct pCIneo/CrFC21. The parent vector pCIneo was digested with NotI, and treated with calf intestinal phosphatase to remove 5' phosphate groups. It was then ligated with full length CrFC21 flanked by NotI ends, derived from an intermediate plasmid construct, pBluescript/ISK/CrFC21. The pCIneo/CrFC21 3'-5' construct containing CrFC21 in the reverse orientation was used as a negative control for subsequent TnT and transfection studies.

also express neomycin phosphotransferase, and can be used for selection of stably expressing transfectants, using the antibiotic G-418.

The vectors for expression of CrFC in mammalian cells carry the cytomegalovirus (CMV) immediate early promoter/enhancer region to promote constitutive expression. They also contain the SV40 early promoter region for induction of transient, episomal replication in cells such as COS-1 or COS-7, expressing the SV40 large T antigen (Gluzman, 1981). The CrFC21 insert

in both constructs contains the native ATG start codon, along with its Kozak consensus element.

In vitro coupled transcription-translation

The recombinant constructs were subjected to T7 promoter driven TnT reactions (Promega, USA), and products analysed as described earlier (Roopashree et al., 1995). In this work, [³H]-leucine (Amersham, UK) was used as the tracer.

Transfection of COS-1 cells

COS-1 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% foetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 10 mM HEPES. CrFC recombinant constructs were isolated using cesium chloride-ethidium bromide gradients (Sambrook et al., 1989). Transfection was carried out by electroporation (Buckler, 1991). Briefly, 10⁷ cells were trypsinised, washed and resuspended in 0.4 ml of PBS, incubated on ice for 10 min with 20-40 µg of DNA and electroporated at 1.2 kV at 25 µF in a cuvette of 0.4 cm path (Gene Pulser, Biorad).

SDS-PAGE and Western analyses

Cells were harvested at 48 and 72 h post-transfection. Cell lysates were prepared by three rounds of freeze-thawing. Fifty µg of the lysate protein was subjected to reducing SDS-PAGE. Gels were electrotransferred to Immobilon PVDF membrane (Millipore), probed with rabbit anti-Factor C antibody, followed by goat anti-rabbit antibody conjugated with horseradish peroxidase, and developed with the substrate mixture of 0.03% 4-chloronaphthol and 3% H₂O₂.

Results and discussion

TnT with pCDNAI/CrFC21 and pCIneo/CrFC21 yielded variable rFCs

The presence and orientation of the full-length CrFC21 insert in pCDNAI/CrFC21 and pCIneo/CrFC21 were verified firstly by restriction analysis, secondly by Southern hybridisation using ³²P-CrFC21 cDNA probe (data not shown), and thirdly by PCR amplification of the CrFC21 fragment from the insert (Roopashree et al., 1996).

Further confirmation of the expression competency was carried out in a TnT assay, before these constructs were utilised for transfection studies. Analysis of the *in vitro* TnT products on SDS-PAGE, followed by fluorography, revealed that pCIneo/CrFC21 was capable of generating the expected polypeptide corresponding to a molecular mass of 113 kDa (Fig. 3a).

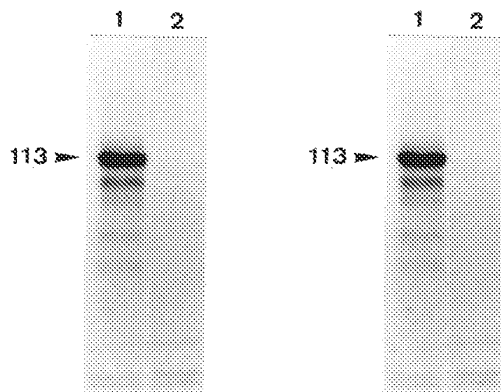


Figure 3 Autoradiograms of TnT products from CrFC21 expression vectors. ^3H -leucine labelled TnT products from (a) pCIneo/CrFC21, and pCIneo/CrFC213'-5' (lanes 1 & 2, respectively) and (b) pCDNAI and pCDNAI/CrFC21 (lanes 1 & 2 respectively). Sizes are indicated in kDa. As expected, the controls pCDNAI and pCIneo/CrFC213'-5' did not generate any product. The minor lower molecular weight bands generated by pCIneo/CrFC21 could be breakdown products of the 113 kDa product. Such an occurrence of breakdown products has also been observed with the Luciferase control DNA that was used to monitor the reaction.

In contrast, the TnT reaction utilising pCDNAI/CrFC21 gave rise to a major product of only 32 kDa, and a minor product of 84 kDa (refer Fig. 3b). Since restriction analysis, Southern hybridisation and PCR have confirmed the presence of full length CrFC21 in pCDNAI, these truncated TnT products could be due to either the recognition of internal start codons at M²³⁸² and M⁶⁶⁶ (Ding *et al.*, 1995), respectively, or the introduction of premature stop codons, following nonspecific point mutations in the insert. These studies have further proven the importance of *in vitro* experiments to verify the integrity of the cDNAs in expression vectors, before further cloning into eukaryotic hosts for *in vivo* expression.

pCIneo/CrFC21-transfected COS-1 cells expressed full length and truncated variants of rFC

COS-1 cells were transfected with recombinant vectors pCIneo/CrFC21, pCIneo/CrFC213'-5' (reverse orientation of insert) and pCDNAI/CrFC21. Transfectants were analysed for expression of rFC at 48 and 72 h post transfection. Cells transfected with pCDNAI/CrFC21 failed to generate rFC. This observation concurred with the earlier TnT assay which generated a major aberrant product of 32 kDa with this construct (Fig. 3).

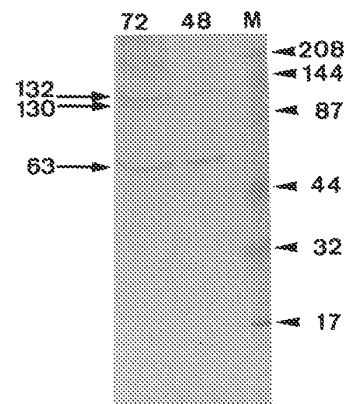


Figure 4 Immunoblot of rFC. The rFC derived from COS-1 cells at 48 and 72 h post transfection, as detected by anti-FC antibodies. The sizes of prestained kaleidoscope (Biorad) molecular weight markers (lane M) are indicated in kDa.

Immunoblots treated with anti-Factor C antibodies revealed that cells transfected with pCIneo/CrFC21 indeed expressed rFC (Fig. 4).

Cells harvested at 48 h post transfection showed the presence of an rFC related protein of 132 kDa, corresponding to full length CrFC21. On the other hand, those harvested at a later time point of 72 h showed the presence of an additional band of 130 kDa, resulting in a closely migrating doublet of rFC proteins. In addition, at both these time points, transfectants also expressed a third band corresponding to an rFC-related protein of 63 kDa.

The presence of rFC proteins of different molecular masses in COS-1 cells indicated that the 132/130 kDa doublet could be a mixture of glycosylated/unglycosylated variants. Such heterogeneity in the recombinant products has been reported in several cases. For example, two electrophoretic variants of murine interleukin-4 were expressed in COS-7 cells that were present as a closely spaced doublet with a molecular weight of 19 kDa (Ramanathan *et al.*, 1989). The same observation has also been reported in the case of human recombinant insulin-like growth factor binding protein (Tressel *et al.*, 1991), recombinant interferon-gamma (IFN- γ , Curling *et al.*, 1989), recombinant t-PA (Moorhouse *et al.*, 1995) and recombinant Factor VIIa (Klausen and Kornfelt, 1995). The smaller rFC related peptide of 63 kDa may be due to the recognition of an internal methionine in the transcript as a start codon for

translation of the polypeptide. Such a feasible start site is M¹⁶⁷ (Ding *et al.*, 1995), where the cDNA stretch ¹⁴¹³AATATGG¹⁴¹⁹ includes a perfect Kozak element.

COS-1 and *P. pastoris* derived rFCs are different in the pattern of their isoforms

Earlier studies on expression of this cDNA in the methylotrophic yeast *Pichia pastoris* (Roopashree *et al.*, 1996), have demonstrated that this yeast can produce significant levels of CrFC transcripts, as well as rFC protein of 130 kDa. On the other hand, the COS-1 derived rFC is comparable to that derived from insect cells, which also generated variants of the 130 kDa species (unpublished data). The present studies indicate that the production of rFC polypeptide differs significantly from lower to higher eukaryotic systems, in the size and number of rFC-related polypeptides. The differences in the patterns of rFC from yeast and COS-1 cells indicates the differences in its trafficking and post-translational modification in these two hosts.

A comparison between rFC and recombinant t-PA

Factor C, although a serine protease zymogen from a lower invertebrate organism, contains several features in its structural organisation (Muta *et al.*, 1991; Iwanaga, 1993), comparable to mammalian complement factors. In fact, it has been suggested that this similarity could be due to a common evolutionary origin for the two systems. This heterologous expression of CrFC could be compared to an extensively studied mammalian blood factor, the t-PA which is also a serine protease that bears a striking resemblance in its structural organisation to CrFC. Although recombinant t-PA from yeast is present in a single chain form (Lemmont *et al.*, 1985), when secreted by Bowes Melanoma cells, it is present in a mixture of glycosylated and unglycosylated forms of 68 and 65 kDa, as well as a cleaved double chain of 35 kDa. This pattern is similar to rFC polypeptides from COS-1 cells, suggesting that the 132 and 130 kDa are glycosylated and unglycosylated species, respectively. The 35 kDa t-PA species, analogous to the 63 kDa band of rFC, is believed to be the two chained form that occurred as a result of specific cleavage between the Arg-Ile sites, by its initiator plasmin present in the culture medium. In the amoebocytes of the horseshoe crabs, CrFC is present in both single and double chain forms (Ding *et al.*, 1993). Upon activation with LPS, the light chain of the latter species is further cleaved into A and B chains of 44 kDa and 8 kDa, respectively. This course of ligand-initiated activation of rFC or t-PA zymogens suggests another

possibility for the occurrence of the 63 kDa rFC band, via the cleavage of the 132 kDa rFC by COS-1 specific cell factors.

The rFC generated from COS-1 cells was associated with the cellular debris. In fact, membrane bound proteins are known to be expressed at much lower levels in COS-1 cells, which are capable of maintaining greater than 100,000 copies of the transfected DNA. Reduction in levels of protein is believed to be due to the lack of membrane surface area for which they can be deposited (Kaufman, 1990), which is perhaps the reason for low levels of rFC expression in COS-1 cells. These studies have however shown the feasibility of production of rFC in mammalian cell culture systems. Further studies on the membrane bound nature of rFC would be necessary to facilitate secretion of this complex molecule in heterologous systems.

Acknowledgements

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References

- Buckler, A.J., Chang, D.D., Graw, S.L., Brook, D.J., Haber, D.A., Sharp, P.A. and Housman, D.E. (1991) *Proc. Natl. Acad. Sci.* 88, 4005-4009.
- Citarella, F., Aiuti, A., La-Porta, C., Russo, G., Peitropoulos, C. and Rinaldi, M. (1992) *Eur. J. Biochem.* 208, 23-30.
- Curling, E.M.A., Hayter, P.M., Baines, A.J., Bull, A.T., Gull, K., *et al.* (1989) *Biochem. J.* 272, 333-337.
- Ding, J.L., Navas III, M.A.A. and Ho, B. (1993) *Biochim. et Biophys. Acta.* 1202, 149-156.
- Ding, J.L., Navas III, M.A.A., Ho, B. (1995) *Mol. Marine Bio. Biotech.* 4, 90-103.
- Gluzman, Y. (1981) *Cell* 23, 175-182.
- Ho, B. (1983) *Microbios Letters* 24, 81-84.
- Iwanaga, S. (1993) *Curr. Opinion Immunol.* 5, 74-82.
- Kaufman (1990) *Methods in Enzymol.* 185, 487-511.
- Klausen, N.K. and Kornfeld, T. (1995) *J. Chromatogr.* 718, 195-202.
- Lemmont, J.F., Wei, C. and Dackowski, W.R. (1985) *DNA* 4, 419-428.
- Moorhouse, K.G., Eusebio, C.A., Hunt, G. and Chen, A.B. (1995) *J. Chromatogr.* 717, 61-69.
- Muta, T., Miyata, T., Misumi, Y., Tokunaga, F., Nakamura, J., Toh, Y., Ikehara, Y. and Iwanaga, S. (1991) *J. Biol. Chem.* 266, 6554-6551.
- Nakamura, T., Morita, T. and Iwanaga, S. (1986) *Eur. J. Biochem.* 154, 511-521.
- Philipova, D., Citarella, F., Venkov, P. and Fantoni, A. (1992) *Yeast* 8, Spec. Iss., S606.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, USA. edition.

- Ramanathan, L., Le, H.V., Labdon, J.E., Mays-Ichino, C.A., Syco, R. and Trotts, P.P. (1989) *Biochim. Biophys. Acta* 1007, 283–288.
- Roopashree, S.D., Chai, C., Ho, B. and Ding, J.L. (1995) *Biochem. Mol. Biol. Intl.* 35, 841–849.
- Roopashree, S.D., Ho, B. and Ding, J.L. (1996) *Mol. Mar. Biol. Biotech.* 5, 344–353.
- Tressel, T.J., Tatsume, G.P., Spratt, K. and Sommer, A. (1991) *Biochem. Biophys. Res. Commun.* 178, 625–633.

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APPENDIX E

1. (currently amended): A method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, ~~secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C having activity of Factor C secreted in the [a]] culture supernatant, wherein the horseshoe crab Factor C is a *Tachyplesus tridentatus* Factor C or a *Limulus polyphemus* Factor C comprising the horseshoe crab Factor C having activity of Factor C,~~

expressing the horseshoe crab Factor B ~~having activity of Factor B~~ in a mammalian cell as a host cell, and recovering a culture supernatant ~~comprising the horseshoe crab Factor B having activity of Factor B.~~

expressing the horseshoe crab Pro-clotting enzyme ~~having activity of Pro-clotting enzyme~~ in a mammalian cell as a host cell, and

~~producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.~~

~~wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.~~

~~and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme and~~

~~formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.~~

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APPENDIX A

Primary Examiner DAVID J STEADMAN
U.S. Patent and Trademark Office
Group Art Unit: 1656
Email: david.steadman@uspto.gov

Re: **Interview Agenda (FOR DISCUSSION PURPOSES ONLY)**
U.S. Application No.: 18/346,420
Inventor: Hikaru MIZUMURA, et al.
Assignee: SEIKAGAKU CORPORATION
Attorney Docket No.: Q289014

Dear Primary Examiner Steadman:

The following are proposals for our telephone interview scheduled for Tuesday, November 14, 2023 at 10 am, which are for your consideration and **for discussion purposes only**.

Proposed Amendment

Claim 1 might be amended as follows:

I. (currently amended): A method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, and recovering ~~the horseshoe crab Factor C having activity of Factor C secreted in a culture supernatant comprising the horseshoe crab Factor C having activity of Factor C,~~

expressing the horseshoe crab Factor B having activity of ~~Factor B~~ in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor B having activity of Factor B,

expressing the horseshoe crab Pro-clotting enzyme having activity of ~~Pro-clotting enzyme~~ in a mammalian cell as a host cell,

~~wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.~~

and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme, and

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~~formulating the endotoxin assay agent comprising the
horseshoe crab Factor C, the horseshoe crab Factor B, and the
horseshoe crab pro-clotting enzyme.~~

Support is found, for example, in paragraph [0075] of the present specification.

Proposed Arguments

§ 112(b) and § 112 (d)

Paragraph [0075] of the specification discloses that "In the endotoxin assay agent of the present invention, the factors and other components may be present as a mixture or present separately. For example, the factors may be mixed at any proportions in formulation, or individual factors may be formulated separately."

The above proposed amendment to claim 1 should overcome the rejections.

§ 103 Rejection

Regarding Ding-1, as the word "culture" indicates, "culture supernatant" refers to an extracellular fraction in culture process which is distinguished from cellular fraction. To further clarify the difference between "a supernatant by centrifuging cultured and lysed host cells" as described in Ding-1 and the claimed "culture supernatant", claim 1 is proposed to be amended to recite "recovering the horseshoe crab Factor C having the activity of Factor C secreted in a culture supernatant".

Ding-1 describes that "Recovery of rCrFC produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, Rather, intracellular rCrFC can be recovered by lysing the mammalian cells with detergents." (Example 1, Ding-1) That is, Ding-1 does not disclose "recovering the horseshoe crab Factor C having the activity of Factor C secreted in a culture supernatant" as defined in amended claim 1.

Regarding Ding-2, Ding-2 does not teach recovering a culture supernatant as recited in amended claim 1.

Before the priority date of this application, it was widely known to those skilled in the art that if mammalian cells are used as a host, Factor C is not secreted extracellularly but remains intracellularly. Specifically, the inventors (Jeak Ling Ding; Bow Ho) of Ding-1 and Ding-2 stated as follows after filing Ding-1 and Ding-2.

"The rFC generated from COS-1 cells was associated with the cellular debris." ." (page 360, right column, lines 4-5 of Roopashree et al, Biotechnology Letters Vol 19, No 4, 1997; "rFC" is abbreviation of "recombinant Factor C"; Cos-1 cell is the mammalian cell exemplified in Ding-1 (column 9, line 41) and Ding-2 (column 20, line 29).)

"Earlier attempts in our lab to express rFC in E. coli, yeast and mammalian cells ... may not have met the requirements for Factor C folding and modification, thus rFCs expressed were sequestered inside these host cells, and only showed weak LPS-binding affinity." (emphasis added.) (page 76, left column, lines 8-14 of Wang et al., Biotechnology Letters 23, 71-76, 2001)

On the other hand, contrary to the above common knowledge, the inventors of this application have discovered that Factor C is secreted extracellularly when CHO cell or HEK cell are used among mammalian cells, as shown in the Examples of this application.

Further, Applicant intends to provide additional experimental data to show that while no Factor C activity was observed in the intracellular fraction prepared based on EXAMPLE 2 of Ding-1, excellent Factor C activity was observed in the culture supernatant as described in the Examples and Fig. 6 of this application. These results demonstrate unexpected effects provided by the claimed feature of "recovering the horseshoe crab Factor C having activity of Factor C secreted in a culture supernatant."

Double Patenting Rejection

Regarding USP 10,982,202, 10,144,923, 11,236,318 and 17/554,128 which are family applications of this application, Applicant intends to submit terminal disclaimer.

Regarding USP 9,040,254, 11,499,177, 17/272,805 and 18/045,705, the claimed invention utilizing culture supernatant of CHO or HEK cell shows unexpected superior effects over Ding-1 and Ding-2.

Hui C. Wauters (Reg. No. 57,426)

APPENDIX B

Factor C Activity in Cell Body Fraction of CHO and HEK cells Expressing Factor C

(1) Expression of LpFC and TtFC in CHO and HEK Cells

In reference to “(2) *Expression of Factor C in Mammalian Cells (CHO DG44)*” sections of the present specification, and WO2018/074498A1, Factor C of *Limulus polyphemus* (LpFC) and *Tachypleus tridentatus* (TtFC) were recombinantly expressed in CHO DG44, CHO-K1, CHO-S and HEK293 cells.

(2) Confirmation of Intracellular Factor C

The cell bodies of CHO-K1, CHO DG44, CHO-S, and HEK293 transformed so as to express LpFC or TtFC were solubilized at room temperature with Sample Buffer Solution without Reducing Reagent (6x) for SDS-PAGE (Nacalai tesque, Japan). The solubilized cells were subjected to SDS-PAGE without purification and subsequent western blotting using anti-Factor C antibody to confirm the presence of intracellular Factor Cs.

As shown in Figure 1A and B, and Figure 2, intracellular Factor Cs were confirmed to be present in CHO-K1, CHO DG44, CHO-S, and HEK cells transformed so as to express LpFC or TtFC.

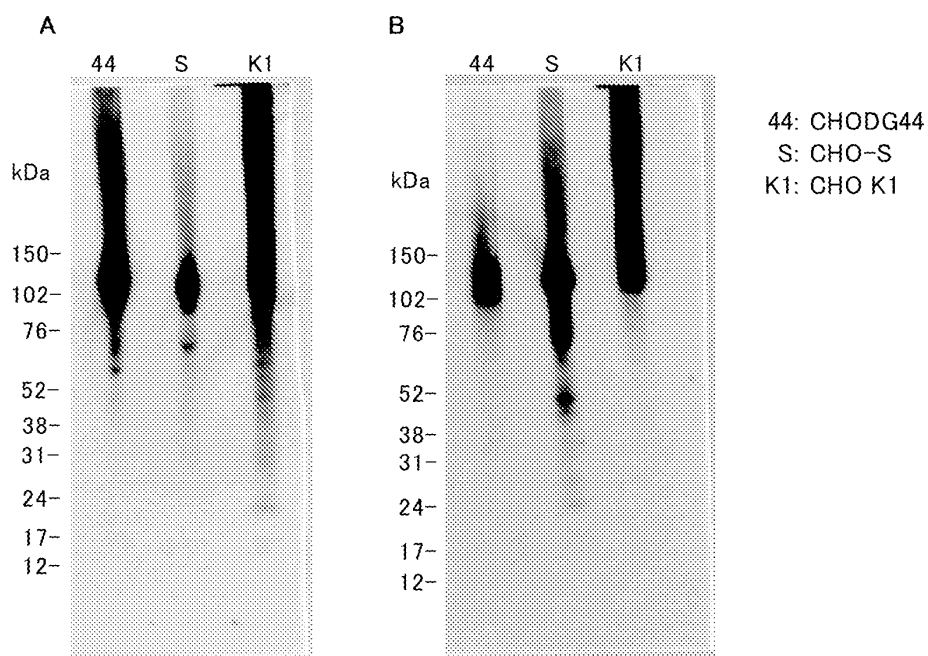


Figure 1: Intracellular LpFC (A) and TtFC (B) of transformed CHO cells; in some samples, the band of Factor C broadened probably due to the impurities arising from cell debris.

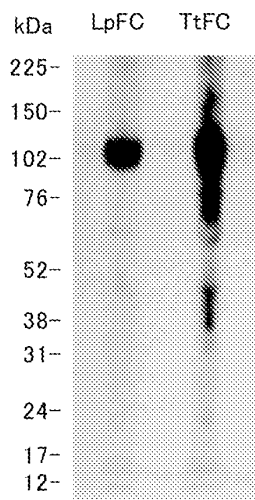


Figure 2: Intracellular LpFC and TtFC of transformed HEK cell; the band of TtFC was broadened probably due to the impurities arising from cell debris.

(3) Measurement of Factor C Activity

Test samples for measuring Factor C activity of intracellular Factor C were prepared according to the procedure disclosed in EXAMPLE 2 of US 5,858,706. Specifically, cell pellet collected by centrifugation was washed with phosphate buffered saline, suspended in breaking buffer (1 w/v % sodium dodecyl sulfate (SDS) in 0.05 M Tris-HCl, pH 8.0, containing 0.1 M NaCl), and solubilized overnight at 4 °C by rotation on an angular rotator. The lysate was cleared by centrifugation, and subjected to membrane filtration (Amicon Ultra-0.5mL Ultracel 30K) for the removal of residual SDS. The retentate was used as cell body fraction (cell lysate) for the following procedures.

Factor C activity was measured in reference to a method disclosed in “4. *Activity Measurement*” section of the present specification. The assay was conducted without endotoxin (blank) or under the presence of 0.1 EU/ml of endotoxin. Factor C activity was calculated as a difference of absorbance change rate between the measured value under the presence of 0.1 EU/ml of endotoxin and blank value. The culture supernatants of CHO, and HEK cells were used as positive controls. The results are summarized in Tables 1 and 2.

As shown in Tables 1 and 2, Factor C activity of intracellular TtFC or LpFC was not detected in the samples prepare as in US 5,858,706.

Table 1: LpFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μ L)	Δ mAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.1
		4	0.0
	Culture supernatant	1	1.1
		2	16.1
		4	16.2
CHO-S	Cell lysate	1	-0.1
		2	0.1
		4	0.1
	Culture supernatant	1	2.5
		2	9.5
		4	12.9
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	11.2
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	7.3

Table 2: TtFC

Host Cell	Cell lysate / Culture supernatant	Amount of Factor C added (μ L)	Δ mAbs/min
CHO DG44	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	0.3
		2	1.8
		4	6.4
CHO-S	Cell lysate	1	0.0
		2	-0.1
		4	0.0
	Culture supernatant	1	0.2
		2	8.5
		4	14.5
CHO K1	Cell lysate	4	0.0
		8	0.0
	Culture supernatant	4	14.3
HEK293	Cell lysate	1	0.0
		2	0.0
		4	0.0
	Culture supernatant	1	9.7

$\Delta mAbs/min = [mAbs/min \text{ at } 0.1 \text{ EU/ml}] - [mAbs/min \text{ at } 0 \text{ EU/ml}]$
[mAbs/min at 0.1 EU/ml]: Absorbance change rate at 0.1 EU/ml of Endotoxin concentration
[mAbs/min at 0 EU/ml]: Absorbance change rate without Endotoxin



Functional expression of full length *Limulus* Factor C in stably transformed Sf9 cells

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Abstract

Limulus Factor C is a potent antagonist of endotoxin from Gram-negative bacteria. A fusion construct containing full length Factor C has been cloned into *Spodoptera frugiperda* Sf9 cell. Stable Sf9 cell transfectants were obtained using Zeocin selection for 2 weeks. The recombinant Factor C (rFC) was secreted into the culture medium at 9 mg l⁻¹. Both the crude and partially purified rFC were able to detect lipid A at 10 pg ml⁻¹ in an ELISA-based lipid A binding assay.

Introduction

Factor C, a serine protease zymogen from the amoebocytes of the horseshoe crab, is sensitive to endotoxin, or lipopolysaccharide (LPS) (Nakamura *et al.* 1986). It initiates the coagulation cascade in the horseshoe crab haemolymph (Nakamura *et al.* 1986) and thus plays a vital role in the defence against invading Gram-negative bacteria. Owing to the high affinity of Factor C for endotoxin (Ding *et al.* 1997, Tan *et al.* 2000), LPS can be detected at fg ml⁻¹ in a modified *Limulus* amoebocyte lysate (LAL) gelation assay (Ho *et al.* 1983). The LAL assay is used widely in the quality assurance of biological and pharmaceutical preparations. However, due to over-harvesting of horseshoe crab for amoebocyte lysate preparations, the species is now endangered. It is therefore imperative to obtain a second generation of 'Limulus amoebocyte lysate' (LAL) by recombinant DNA technology.

Expression of recombinant Factor C, rFC, in heterologous hosts have been carried out using various systems: *E. coli* (Roopashree *et al.* 1995), *Pichia pastoris* (Ding *et al.* 1996), *Saccharomyces cerevisiae* (Roopashree *et al.* 1997b), and COS-1 (Roopashree *et al.* 1997a). However, none of these recombinant proteins were secreted and they remained insoluble

within the host cells, indicating that the native signal sequence was not able to direct full length rFC out of these heterologous hosts.

In the current study, a new approach for production and secretion of rFC is carried out. Our laboratory has recently developed a strong novel secretory signal, ss (US Patent filed). By replacing the native signal peptide of CrFC21 with ss, we are able to obtain soluble rFC in the culture supernatant. *Spodoptera frugiperda* (Sf9) cell was chosen as the expression host, since it is genetically closer to arthropod than any other heterologous hosts used in earlier experiments. We describe here the expression pattern of the full-length Factor C in stably transformed Sf9 cells, and the functionality of rFC is also investigated.

Materials and methods

All restriction and DNA modifying enzymes were purchased from Boehringer Mannheim and New England Biolabs.

Construction of expression plasmid

Lepidopteran insect expression vector, pIZT/V5-His (Invitrogen) was used for fusion expression. The na-

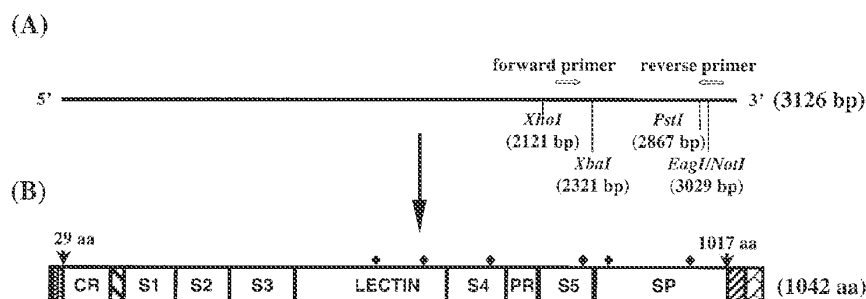


Fig. 1. (A) Recombinant Factor C cDNA. Primers used for PCR are presented by open arrows ($\Rightarrow$). Restriction sites used for expression vector construction and Southern blot probe preparation are also indicated. (B) Domain structure of the recombinant Factor C. The fusion sites at N- and C-terminus are indicated by closed arrows ($\downarrow$) with the amino acids numbered corresponding to the original CrFC21 (GenBank Accession No: S77063). The potential glycosylation sites are marked with diamonds ($\blacklozenge$). $\square$: Secretory signal (ss); CR: cysteine-rich region; $\square$: epidermal growth factor-like domain; S1–5: sushi-like domains 1–5; LECTIN: lectin-like domain; PR: proline-rich region; SP: serine-protease coding region; $\square$: V5 epitope; $\square$: 6 $\times$ His tag.

tive signal peptide sequence of full length cDNA, CrFC21 (Ding *et al.* 1995), was replaced by a novel secretion signal, ss, to direct the fusion protein out of the host cells. The cDNA fragment containing the entire open reading frame was obtained by digestion with *KpnI* and *NotI* from pBluescript II SK vector (Stratagene), and inserted into compatible sites in pIZT/V5-His. A pair of primers (forward: 5'-GATGGATCCCTCGTTCTCCTTTTATC-3'; reverse: 5'-ATCGGCCCGCCTAATCCATGACAGG-3') with *BamHI* and *EagI* sites (underlined) were used to obtain the 786 bp 3'-terminal portion of Factor C cDNA (Figure 1A). PCR was performed with a hot start at 94 °C for 2 min, followed by 30 cycles of two-min denaturation at 94 °C, 1 min of annealing at 60 °C, and 1 min of extension at 72 °C. This fragment was subsequently digested with *XbaI* and *EagI* to replace the *XbaI-NotI* fragment in native Factor C cDNA, allowing the removal of stop codon as well as in-frame fusion between the cDNA and V5-epitope/6 $\times$ His tag in the expression vector. Figure 1B depicts the domain structures of the recombinant construct. In pIZT/ssFLFC-V5-His, 29–1017 aa of CrFC21 (Ding *et al.* 1995) was fused between ss at the N-terminus end and V5-epitope/6 $\times$ His tag at the C-terminus. In this construct, the recombinant full length Factor C cDNA was placed under transcriptional control of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus immediate-early 2 gene promoter, OpIE2 (Theilmann & Steward 1992). In addition, it contains a Zeocin resistance gene for selecting transformants in Zeocin-containing growth medium. The construct

was transformed into competent *E. coli* TOP10F⁺, and the DNA sequence was verified by BigDye Terminator Cycle Sequencing system (Perkin-Elmer, ABI Prism377).

In vitro coupled transcription-translation (TnT)

To ensure the expression competency of the construct, the cDNA insert was digested by *KpnI* and *EagI*, and recloned into compatible sites in pBluescript II SK vector containing T7 promoter. The resultant recombinant construct, pBS/ssFLFC-V5-His, was subjected to TnT reaction (Promega) in standard volumes of 50 μ l each containing 2 μ g of plasmid DNA. [³H]-Leucine (Amersham, UK) was used to monitor the efficiency of the reaction which was carried out at 30 °C for 90 min. The TnT products were resolved by 10% (w/v) SDS-PAGE and the gel was stained with Coomassie Blue for 30 min, fluorographed for 30 min with Amplify (Amersham), dried and autoradiographed for 16 h at –70 °C. Autoradiograms were developed using a Kodak X-Omat processor.

Cell culture and transfection

Sf9 insect cells were maintained in Sf-900 II SFM (Gibco) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Clontech), 50 U penicillin ml^{–1} and 50 μ g streptomycin ml^{–1}. The cells were incubated as monolayers at 27 °C in a humidified incubator. Two ml cells, 1 $\times$ 10⁶ cells ml^{–1}, in 35 mm wells (Iwaki) were transfected with 3.3 μ g CsCl-purified pIZT/ssFLFC-V5-His DNA mediated

by 6.7 μ l Insectin-Plus (Invitrogen) in 200 μ l Sf-900 II SFM without supplement and antibiotics. After transfection, cells were maintained in the DNA-lipid complex for 5 h. Subsequently, the solution was removed and replaced by 2 ml fresh medium. Cells were allowed to recover for 2 days before selection. Stable transfectants were selected with Zeocin (Invitrogen) at 1000 μ g ml⁻¹ for 2 weeks. Stable cell lines expressing rFC were adapted to Sf-900 II SFM by weaning over four to five passages. Subsequently, Sf9 transfectants were cultured in suspension in a 250-ml spinner flask (Bellco, USA) containing 100 ml Sf-900 II SFM, and further scaled up to a 500-ml Bellco spinner flask containing 200 ml medium. Cell cultures were harvested at mid-growth phase at $3\text{--}4 \times 10^6$ cell ml⁻¹. All procedures were carried out under pyrogen-free conditions.

Genomic DNA analyses

Total genomic DNA was isolated from Sf9 stable transfectants using InstaGene Matrix (BioRad) according to the manufacturer's instructions. To confirm the integration of the pIZT/ssFLFC-V5-His in the Sf9 cell genome, the region corresponding to the 3' terminal portion of CrFC21 cDNA was amplified using specific forward and reverse primers as described. The PCR-derived product was used for Southern blotting. An *Xho*I-*Pst*I fragment located at the 3'-terminus of CrFC cDNA was [³²P] labelled and used as the specific probe (Figure 1A).

Western analyses of intracellular and secreted rFC proteins

The three protein fractions of interest are defined as: (1) M: protein secreted into medium, which was obtained by clarification of conditioned medium at 14000 $\times g$ for 5 min at 4 °C; (2) L: soluble cell lysate derived from cells of 2 ml culture that was lysed in 100 μ l PBS by 5 cycles of freeze-thawing at -70 °C and 37 °C, followed by centrifugation at 14000 $\times g$ for 15 min at 4 °C; and (3) P: the resultant insoluble fraction containing particulate proteins. Fraction P was solubilised in 100 μ l SDS-PAGE sample buffer containing 50 mM Tris/HCl, pH 6.8, 2% (w/v) SDS and 2% (v/v) β -mercaptoethanol. The total soluble protein in medium (M) and cell lysate (L) was measured using Bradford method. Western analyses of the rFC proteins using anti-V5-HRP antibody (Invitrogen) was carried out. The blots were visualised using the Supersignal West Pico Chemiluminescent

Substrate (Pierce). The relative amounts of rFC in the three fractions were quantitated densitometrically by using ImageMaster (Pharmacia) software. Recombinant Factor C production was estimated in comparison to a purified protein containing V5 epitope in Western blot analysis.

Concentration and partial purification of secreted rFC samples

Culture supernatant was collected after centrifugation of cell culture at 3000 $\times g$ for 10 min at 4 °C to remove all the cells. The supernatant was concentrated 10 times by ultrafiltration using regenerated cellulose membrane cassette (Pellicon XL, 30 kDa molecular weight cut-off, MWCO, Millipore). Partial purification was carried out by gel filtration chromatography through Sephadex G-100 (1.5 $\times$ 90 cm), using 0.05 M Tris/HCl (pH 7.5), 0.154 M NaCl as running buffer. Fractions of 1 ml were collected, and the fractions corresponding to the void peak were pooled and concentrated using Centrprep regenerated cellulose membrane (30 kDa MWCO, Amicon YM).

Endotoxin-binding analysis

The LPS-binding potential of the secreted soluble rFC protein was analysed by an ELISA-based assay for lipid A, the biologically active component of LPS.

One hundred μ l of diphosphoryl lipid A from *E. coli* F-583 (Sigma) of different concentrations were coated on the wells of a polysorp 96-well plate (Nunc) by incubating at 37 °C for 2 h. The wells were washed 3 times with 200 μ l of wash solution [pyrogen-free PBS containing 0.01% (v/v) Tween-20, 0.01% (w/v) merthiolate], and blocked overnight at room temperature with pyrogen-free PBS containing 0.2% (w/v) BSA, and 0.01% (v/v) Tween-20. After 3 washes, 100 μ l samples containing 10 μ g protein was added into each well, followed by 16 h of incubation at room temperature. The samples were removed and the wells were washed 3 times. Subsequently, 100 μ l rabbit anti-Factor C antibody (1:500 dilution) was added and incubated for 3 h at 37 °C followed by 3 washes before addition of 100 μ l goat anti-rabbit secondary antibody (1:2000 dilution) conjugated to horseradish peroxidase (Dako). Finally, 100 μ l ABTS solution (Boehringer Mannheim) was used as substrate. The absorbance of the samples was determined at 405 nm with reference wavelength at 490 nm using the SPECTRAmax 340 plate reader, with SOFTmax PRO version 1.2.0.

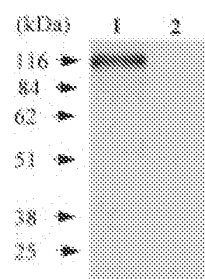


Figure 2. Autoradiogram of TnT product from pBS/ssFLFC-V5-His expression construct. Ten μ l of the total reaction mix (50 μ l) was loaded onto each lane of 10% (w/v) SDS polyacrylamide gel under reducing conditions. ^{3} H]-Leucine labelled TnT products from pBS/ssFLFC-V5-His (lane 1) and pBluescript II SK vector DNA as negative control (lane 2).

Results

Construction of pIZT/ssFLFC-V5-His and verification of expression competency

After removal of the native signal sequence and stop codon, the CrFC cDNA was inserted between a novel secretory signal sequence and 3'-end V5 epitope and 6 $\times$ His tag coding sequence, resulting in pIZT/ssFLFC-V5-His. This recombinant plasmid, therefore, contains the native CrFC sequence encoding amino acid, 29-1017. To confirm the efficacy of the start codon recognition, and to rule out the existence of any premature stop codon introduced during the cloning process, TnT (*in vitro* coupled transcription translation) analyses were carried out. The cDNA insert from pIZT/ssFLFC-V5-His was re-cloned into the T7 promoter containing vectors which allowed TnT experiments. The expression construct, pBS/ssFLFC-V5-His was thus obtained. The presence and orientation of the inserts were verified by restriction analysis and sequencing. The 3126 bp cDNA insert generated a 116 kDa polypeptide corresponding to its calculated molecular mass (Figure 2). The absence of other minor bands indicates strong and specific recognition of start codon and correct termination of translation in the reticulocyte lysate system. The integrity of the original cDNA in pIZT/V5-HisA was thus verified and used for *in vivo* expression studies in Sf9 cells.

Genomic DNA analysis

After two weeks of selection, total DNA from the stable Sf9 cell transfectants was extracted and used as template for PCR to test for the integration of the

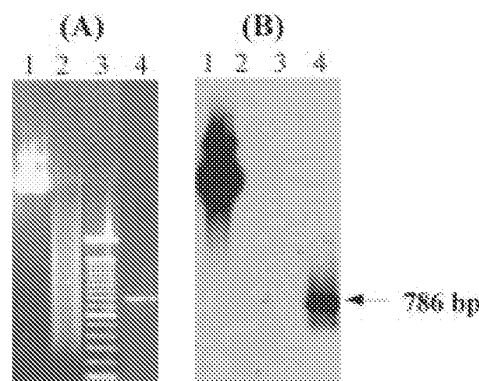


Figure 3. PCR and Southern blot analysis of integration of Factor C cDNA into Sf9 cell genome. (A) Electrophoresis of PCR product in 1% (w/v) agarose gel. Lane 1: positive control: pIZT/ssFLFC-V5-His plasmid DNA; lane 2: negative control: PCR product from wild type Sf9 cell genomic DNA; lane 3: molecular weight standards: 100 bp ladder DNA marker (Gibco BRL); lane 4: PCR product from stable Sf9 transfectant genomic DNA. Primers used for PCR are indicated in Figure 1. (B) Southern hybridization of PCR fragments. The gel was Southern blotted and probed with ^{32} P] labelled *XhoI-PstI* fragment (the 3' end of CrFC21; see Figure 1A)

expression vector. The PCR product of 786 bp was generated only from the transfected Sf9 cell DNA (Figure 3A). Southern analysis of this product showed this fragment hybridized strongly and specifically with the homologous ^{32} P] CrFC21/*XhoI-PstI* probe (Figure 3B). It indicates that the rFC gene was indeed intact and the expression cassette was inserted into the Sf9 cell genome.

Transfected Sf9 cells expressed full length rFC

By using anti-V5-HRP antibody as probe, rFC was detected in transient transfection studies as early as 24 h post-transfection (data not shown). This indicates a successful readthrough of the CrFC sequence into the V5-His fusion tag. The immunoreactive rFC was 132 kDa in size (Figure 4), compared to the 116 kDa band observed by TnT. It indicates that extensive post-translational modifications were carried out in the host Sf9 cell. Furthermore, the intracellular rFC was observed in both soluble and insoluble fractions. These intracellular rFCs had lower mobility in the blot than the extracellular rFC, which indicates the cleavage of the signal peptide and glycan modification during the secretory pathway. The majority of rFC protein was secreted into the culture medium. According to densitometric scan, 84% of the total rFC protein was present

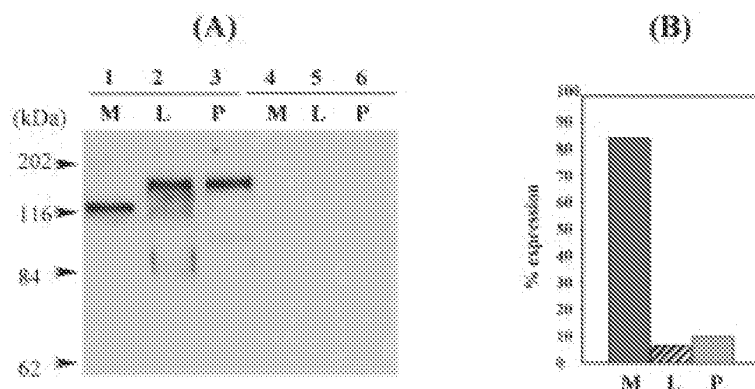


Figure 4. (A) Western blot analysis of protein samples from M (culture medium, 1.0 μ g), L (soluble cell lysate, 10 μ g) and P (insoluble fraction, 10 μ g). Lanes 1, 2, 3: protein samples from Sf9 stable transfectant, lanes 4, 5, 6: protein samples from wild type Sf9 cells. Sizes of molecular markers are indicated at the left. (B) Percentage of rFC present in three fractions, M (84%), L (6%) and P (10%). Since M contains 84% of expressed rFC, only 1.0 μ g of secreted proteins was used in the Western blot to assure the exposure was within the linear range of film and scanner response.

in the culture supernatant, while only 6% and 10% of the recombinant protein were localized in the soluble cell lysate (L) and insoluble particulate fraction (P), respectively. By comparison with a purified protein (> 99% purity) containing V5-epitope in Western blot, the expression level of rFC in culture supernatant was determined to be 9 mg l^{-1} .

The His-antibody unexpectedly failed to detect recombinant protein in immunoblotting. The cleavage of His-tag is probably due to proteolysis of the carboxyl end of the recombinant proteins, by intra- or extra-cellular proteases.

Secreted rFC is functional

The rFC from Sf9 cells was tested for their functionality using ELISA (Figure 5). A broad range of concentration of lipid A was used in the assay. In lipid A-based ELISA, rFC protein showed apparent binding compared to the control medium. As low as 10 pg ml^{-1} lipid A was detected in the assay. As the concentration of lipid A was increased, we consistently obtained increasing OD readings of the ABTS substrate. Gel filtration enriched the rFC proteins, resulting in 33% increase in binding, indicating higher sensitivity to lipid A after removal of the small size molecules and ions, which may interfere with the binding.

Although intracellular rFC was also obtained in this system, it only accounts for a small percentage of total rFC expressed, and due to numerous endoge-

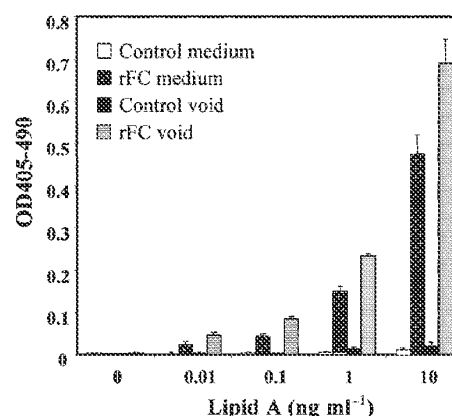


Figure 5. ELISA-based lipid A binding assay of rFC samples from Sf9 cells. Ten μ g protein samples were used in the assay. Control medium: concentrated serum free medium from untransfected Sf9 cells; rFC medium: concentrated serum free medium from stable Sf9 cell line expressing rFC; control void: protein sample concentrated from the void fraction of control medium passed through Sephadex G-100 column; rFC void: protein sample concentrated from the void fraction of rFC medium passed through Sephadex G-100 column.

nous proteins, additional steps may be necessary to yield biologically active rFC, and thus limits its application value. Thus the 84% secreted and functional rFC offers an advantageous alternative to LAL.

Discussion

Factor C is a large complex multi-domain protein of 132 kDa, and has six potential *N*-glycosylation sites. As delineated by earlier studies, the *N*-terminus of Factor C contains intricately folded cysteine-rich and EGF-like domains followed by the important LPS-binding domain. Further downstream, there is a proline-rich region which shares homology with the hinge region of human coagulation Factor XII. Earlier attempts in our lab to express rFC in *E. coli*, yeast and mammalian cells (Roopashree *et al.* 1995, 1996, 1997, Ding *et al.* 1997) may not have met the requirements for Factor C folding and modification, thus rFCs expressed were sequestered inside these host cells, and only showed weak LPS-binding affinity (Ding *et al.* 1997, Pui *et al.* 1997, 1998).

In this work, we have used Sf9 cell as a host for expression of rFC, and a new approach was employed to create stable cell lines that continuously produce the recombinant protein of interest. This method differs from the conventional baculovirus expression system in that non-lytic, long-term stable expression is allowed without viral infection at every cycle of expression, so that significant time and effort is saved. A stable polyclonal population of Sf9 transformant was obtained in only two weeks. In our case, the expression level of rFC remained constant over 30 passages without Zeocin selection.

By introducing the novel secretory signal (ss) into Factor C, we successfully detected the protein in the culture supernatant. The expression level we observed is relatively high (9 mg l⁻¹). It is comparable to other recombinant proteins expressed under the OpIE2 promoter (Hegedus 1999). Fusion of C-terminal epitope allows easy detection of the rFC. The unexpected failure of detecting rFC utilizing 6 × His tag Probe indicates the trimming of the full length rFC, suggesting employment of alternative tag for detection and purification of the rFC, for example, 10 × His tag.

As shown by ELISA, the soluble and secreted form of rFC is functional in specifically recognising and binding lipid A. Sf9 cell itself secretes only low level of endogenous protein, thus reducing the steps required for its purification. The assay shows that rFC is of practical value in detecting trace amounts of LPS.

In conclusion, we have utilised a new approach for the production of rFC. The secreted Factor C conforms to the expected size, is immunoreactive and biologically active. This system offers advantages for the production of rFC, due to its high expression, ca-

pability of scale-up culture in bioreactors, efficient secretion and ease of purification. This recombinant Factor C may substitute conventional 'LAL' assay for mass detection of LPS in the near future.

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References

- Ding JL, Chai C, Pui WM, Ho B (1997) Expression of full length and deletion homologues of *Carcinoscorpius rotundicauda* Factor C in *Saccharomyces cerevisiae*: immunoreactivity and endotoxin binding. *J. Endotoxin Res.* 4: 33–43.
- Ding JL, Navas MAIII, Ho B (1995) Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Carcinoscorpius rotundicauda*. *Mol. Mar. Biol. Biotech.* 4: 90–103.
- Ding JL, Tan NS, Ho B, Lam TJ (1996) Isolated nucleic acids encoding a secretory signal for expression and secretion of heterologous recombinant proteins. US Patent Application No. 09/426, 776, PCT/SG99/00108, WO 00/26366.
- Hegedus DD, Pfeifer TA, Theilmann DA, Kennard ML, Gabathuler R, Jefferies WA, Griglati TA (1999) Differences in the expression and localization of human melanotransferrin in lepidopteran and dipteran insect cell lines. *Protein Express. Purif.* 15: 296–307.
- Ho B (1983) An improved *Limulus* gelation assay. *Microbios. Lett.* 24: 81–84.
- Nakamura T, Morita T, Iwanaga S (1986) Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in *Limulus* hemocytes. Isolation and characterization. *Eur. J. Biochem.* 154: 511–521.
- Pui AWM, Ho B, Ding JL (1997) Yeast recombinant Factor C from horseshoe crab binds endotoxin and causes bacteriostasis. *J. Endotoxin Res.* 4: 391–400.
- Pui AWM, Roopashree SD, Ho B, Ding JL (1998) Recombinant Factor C from *Carcinoscorpius rotundicauda* binds endotoxin. In: *New Development in Marine Biotechnology*. New York: Plenum Press, pp. 151–154.
- Roopashree SD, Chai C, Ho B, Ding JL (1995) Expression of *Carcinoscorpius rotundicauda* Factor C cDNA. *Biochem. Mol. Biol. Intl.* 35: 841–849.
- Roopashree SD, Ho B, Ding JL (1996) Expression of *Carcinoscorpius rotundicauda* Factor C in *Pichia pastoris*. *Mol. Mar. Biol. Biotech.* 5: 334–343.
- Roopashree SD, Ho B, Ding JL (1997a) Recombinant COS-1 cells express *Carcinoscorpius rotundicauda* Factor C. *Biotechnol. Lett.* 19: 357–361.
- Roopashree SD, Ho B, Ding JL (1997b) The cys-rich and EGF-like domains of *Carcinoscorpius rotundicauda* Factor C yields soluble fusion protein with GFP. *Biotechnol. Lett.* 19: 1147–1150.
- Tan NS, Ho B, Ding JL (2000) High-affinity LPS binding domain(s) in recombinant Factor C of a horseshoe crab neutralizes LPS-induced lethality. *EASEB J.* 14: 859–870.
- Theilmann DA, Steward S (1992) Molecular analysis of the transactivating IE-2 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* 187: 84–96.



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Art Unit	1656
Examiner Name	DAVID J STEADMAN
Practitioner Docket No.	Q289014

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Title of Invention:	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN
First Named Inventor/Applicant Name:	Hikaru MIZUMURA
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DETAILED CORRESPONDENCE

Status of the Application

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1-10 are pending in the application.

Priority

This application is a continuation application of U.S. non-provisional application no. 17/554,128, filed on December 17, 2021, which is a continuation application of U.S. non-provisional application no. 16/399,386, filed on April 30, 2019, now U.S. Patent No. 11,236,318, which is a continuation application of U.S. non-provisional application no. 15/983,725, filed on May 18, 2018, now U.S. Patent No. 10,982,202, which is a continuation application of U.S. non-provisional application no. 14/650,767, filed on June 9, 2015, now U.S. Patent No. 10,144,923, which is filed under 35 U.S.C. 371 as a national stage of international application PCT/JP2013/083082, filed on December 10, 2013, which claims foreign priority under 35 U.S.C. 119(a)-(d) to Japanese application no. 2012-269840, filed on December 10, 2012. A certified copy of the foreign priority document has been filed in the prior-filed application no. 14/650,767 on June 9, 2015.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on July 3, 2023 and August 18, 2023 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the IDSs are being considered by the examiner.

Cite Nos. 20, 23, 24, 27, 29, and 80 of the IDS filed on July 3, 2023 have been lined through because, according to the applicant's "Submission of a Corrected Form PTO/SB/08" filed on August 18, 2023, these cites contain errors, which are corrected by the IDS filed on August 18, 2023.

Claim Objections

Claim 4 is objected to in the recitation of "*Tachypleus tridentatus*, and *Limulus polyphemus*" and in the interest of improving claim form, it is suggested that the comma be deleted from the noted phrase.

Claim Rejections - 35 USC § 112(b)

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

Claim 9 is rejected under 35 U.S.C. 112(b) as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor regards as the invention.

Claim 9 is indefinite in the recitation of "wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are formulated separately", which is inconsistent with the recitation of "formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme" in claim 1. It is suggested that the applicant clarify the meaning of claim 9.

Claim Rejections - 35 USC § 112(d)

The following is a quotation of 35 U.S.C. 112(d):

(d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim 8 is rejected under 35 U.S.C. 112(d) as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Given that claim 1 recites “formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme”, claim 8 does not further limit claim 1. Applicant may cancel the claim, amend the claim to place the claim in proper dependent form, rewrite the claim in independent form, or present a sufficient showing that the dependent claim complies with the statutory requirements.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-10 are rejected under 35 U.S.C. 103 as being unpatentable over Ding-1 et al. (US 5,858,706; cited on the IDS filed on July 3, 2023; hereafter “Ding-1”) in view of

Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on July 3, 2023; hereafter “Tamura”).

The claims are drawn to a method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor C having activity of Factor C,

expressing the horseshoe crab Factor B in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor B having activity of Factor B,

expressing the horseshoe crab Pro-clotting enzyme in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme, and

formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.

The specification does not appear to define the phrase “culture supernatant” and given a broadest reasonable interpretation, the phrase “culture supernatant” is interpreted as encompassing a supernatant recovered by centrifuging cultured and lysed host cells.

Regarding claim 1, the reference of Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to

express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant by centrifuging cultured and lysed host cells (column 10, lines 43-47).

Regarding claim 4, Ding-1 teaches the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Regarding claim 5, Ding-1 does not teach the Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that *T. tridentatus* factor C polypeptide

recombinantly expressed and recovered from CHO cells according to Ding-1 also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, Ding-1 does not teach the Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

The differences between Ding-1 and the claimed invention are:

- 1) Ding-1 does not teach recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1; and
- 2) Ding-1 does not teach filtration of a supernatant as recited in claim 7.

Regarding difference 1), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-1 for an endotoxin assay reagent with Ding-1's recombinantly produced *Tachypleus tridentatus* Factor C. One would have been motivated to do this because Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme. One would have had a reasonable expectation of success to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-1 for an endotoxin assay reagent with Ding-1's recombinantly produced *Tachypleus tridentatus* Factor C because Tamura taught sequences of *Tachypleus tridentatus* Factor B and Pro-clotting proteins, and Ding-1 and Tamura taught methods for recombinant expression of a protein using a CHO cell.

Regarding difference 2), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Ding-1 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, the method for producing an endotoxin assay agent of claims 1-10 would have been obvious to one of ordinary skill in the art before the effective filing date.

Claims 1-10 are rejected under 35 U.S.C. 103 as being unpatentable over Ding et al. (US 5,716,834; cited on the IDS filed on July 3, 2023; hereafter “Ding-2”) in view of Tamura.

The specification does not appear to define the phrase “culture supernatant” and given a broadest reasonable interpretation, the phrase “culture supernatant” is interpreted as encompassing a supernatant obtained by recovering a culture broth from cultured host cells.

Regarding claims 1 and 4, Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster

Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Regarding claim 5, Ding-2 does not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Factor C polypeptide recombinantly expressed and recovered from CHO cells according to Ding-1 also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, Ding-2 does not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

The differences between Ding-2 and the claimed invention are:

- 1) Ding-2 does not teach recovering a culture supernatant as recited in claim 1;
- 2) Ding-2 does not teach recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1; and
- 3) Ding-2 does not teach filtration of a supernatant as recited in claim 7.

Regarding difference 1), Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to collect the culture medium following secretion of Factor C according to Ding. One would have been motivated to do this in order to isolate or harvest the Factor C following secretion. One would have had a reasonable expectation of success to do this because Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of

endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-2 for an endotoxin assay reagent with Ding-2's recombinantly produced *Tachypleus tridentatus* Factor C. One would have been motivated to do this because Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme. One would have had a reasonable expectation of success to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-2 for an endotoxin assay reagent with Ding-2's recombinantly produced *Tachypleus tridentatus* Factor C because Tamura taught sequences of *Tachypleus tridentatus* Factor B and Pro-clotting proteins, and Ding-2 and Tamura taught methods for recombinant expression of a protein using a CHO cell.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, the method for producing an endotoxin assay agent of claims 1-10 would have been obvious to one of ordinary skill in the art before the effective filing date.

Claim Rejections - Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory

double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 8 of U.S. Patent No. 10,982,202 B2 (Cite No. 9 of the IDS filed on July 3, 2023; hereafter “patent”) in view of Tamura.

The claim of the patent does not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application;
- 3) filtration of a supernatant as recited in claim 7 of this application; and
- 4) a method for measuring endotoxin as recited in claim 10 of this application.

Regarding difference 1), Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is considered to be “recovering a culture supernatant”.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects

and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught collecting the culture medium of a protein that is secreted into a culture medium, Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins, and Tamura taught that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would

have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Regarding difference 4), Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to use the endotoxin assay agent of the claims of the patent, including recombinantly-produced Factor B, and recombinantly-produced Pro-clotting enzyme, to detect endotoxin. One would have been motivated to and would have had a reasonable expectation of success to do this because the claims of the reference application recite an endotoxin assay agent comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme, and Tamura taught detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claim 8 of the patent in view of Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 8, and 9 of U.S. Patent No. 10,144,923 B2 (Cite No. 2 of the IDS filed on July 3, 2023; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application; and
- 3) filtration of a supernatant as recited in claim 7 of this application.

Regarding difference 1), Ding-1 teaches recovery of the Factor C from mammalian cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47), *i.e.*, “recovering a culture supernatant”. Ding-2 teaches the alternative of secreting Factor C into a culture medium (column 3, lines 47-50; column 17, lines 24-28) and Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (*e.g.*, SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (*e.g.*, SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1, 3, 8, and 9 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 4, and 10-12 of U.S. Patent No. 11,236,318 B2 (Cite No. 10 of the IDS filed on July 3, 2023; hereafter "patent") in view of in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application; and
- 3) filtration of a supernatant as recited in claim 7 of this application.

Regarding difference 1), Ding-1 teaches recovery of the Factor C from mammalian cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47), *i.e.*, "recovering a culture supernatant". Ding-2 teaches the alternative of secreting Factor C into a culture medium (column 3, lines 47-50; column 17, lines 24-28) and Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be "recovering a culture supernatant".

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (*e.g.*, SEQ ID NO: 15 and 16 of Tamura)

and Pro-clotting enzyme (*e.g.*, SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 2, 4, and 10-12 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1 and 3-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,040,254 B2 (cited on the attached Form PTO-892; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and

2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the

Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the patent do not recite the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, the claims of the patent do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1 and 3-10 of this application are unpatentable over claim 1 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 and 7-9 of U.S. Patent No. 11,499,177 B2 (cited on the attached Form PTO-892; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and
- 2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin

testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1,

Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the patent do not recite the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, the claims of the patent do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128

kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1 and 7-9 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-6, 8, and 9 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application).

Regarding claims 1-4 and 8 of this application, claim 3 of the reference application recites an endotoxin assay agent comprising a horseshoe crab Factor C protein having an activity of Factor C, a horseshoe crab Factor B protein having an activity of Factor B, and a horseshoe crab Pro-clotting enzyme protein having an activity

of Pro- clotting enzyme, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus* and *Limulus polyphemus*,

wherein the Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell,

wherein the Factor B protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell,

wherein the Pro-clotting enzyme protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell, and

wherein the endotoxin assay agent dose not comprise a native Factor C,
claim 15 of the reference application recites wherein the Factor C protein is secreted into a culture supernatant and recovered from the culture supernatant,

claim 16 of the reference application recites wherein the Factor B protein is secreted into a culture supernatant and recovered from the culture supernatant, and

claim 17 of the reference application recites wherein the Pro-clotting enzyme protein is secreted into a culture supernatant and recovered from the culture supernatant.

Regarding claim 5 of this application, claim 5 of the reference application recites wherein the Factor C protein contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

Regarding claim 6 of this application, claim 6 of the reference application recites wherein the Factor C protein has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant provisional rejection.

Therefore, claims 1-6, 8, and 9 of this application are unpatentable over claims 2, 3, 5, and 15-17 of the reference application.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 7 and 10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application) in view of Tamura.

The claims of the reference application do not recite:

- 1) filtration of a supernatant as recited in claim 7 of this application; and
- 2) a method for measuring endotoxin as recited in claim 10 of this application.

Regarding difference 1), Tamura teaches a recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Regarding difference 2), Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to use the endotoxin assay agent of the claims of the reference application to detect endotoxin. One would have been motivated to and would have had a reasonable expectation of success to do this because the claims of the reference application recite an endotoxin assay agent comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme, and Tamura taught detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme.

Therefore, claims 7 and 10 of this application are unpatentable over claims 2, 3, 5, and 15-17 of the reference application in view of Tamura.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3 of co-pending application 17/272,805 (reference application) in view of Ding-1, Ding-2, and Tamura.

The claims of the reference application do not recite:

1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and

2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the *Limulus* amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of *Limulus* amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2

discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachyplesus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the reference application do not recite the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a

horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, the claims of the reference application do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced Factor C, Factor B,

and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1-3 of the reference application in view of Ding-1, Ding-2, and Tamura.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1 and 7-9 of co-pending application 18/045,705 (reference application) in view of Ding-1, Ding-2, and Tamura.

The claims of the reference application do not recite:

- 1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and
- 2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin

detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of *Limulus* amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2

discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the reference application do not recite the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T.*

tridentatus factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 6, the claims of the reference application do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1 and 7-9 of the reference application in view of Ding-1, Ding-2, and Tamura.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Status of the claims:

Claims 1-10 are pending.

Claims 1-10 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Application/Control Number: 18/346,420
Art Unit: 1656

Page 40

/David Steadman/
Primary Examiner, Art Unit 1656

<i>Notice of References Cited</i>	Application/Control No. 18/346,420		Applicant(s)/Patent Under Reexamination MIZUMURA et al.	
	Examiner DAVID J STEADMAN		Art Unit 1656	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-9040254-B2	05-2015	Tamura; Hiroshi	C12Q1/56	435/23
*	B	US-11499177-B2	11-2022	Kobayashi; Yuki	C12Y304/21085	1/1
	C					
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	E					
	F					
	G					
	H					
	I					
	J					
	K					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

U.S. Patent and Trademark Office
PTO-892 (Rev. 01-2001)

Notice of References Cited

Part of Paper No. 20230911

<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
PATENT DATA PORTAL inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
SEARCH inventor name and assignee search, all inventors searched	09/12/2023	/DJS/
Reviewed prosecution history for priority applications 14/650,767, 15/983,725, 16/399,575, 16/399,386, 17/554,128	09/12/2023	/DJS/
SEARCH, STN database keyword search, see attached search printouts for search strategy and databases searched	09/12/2023	/DJS/
GOOGLE keyword search, see attached search printouts for search strategy	09/12/2023	/DJS/
Reviewed Global Dossier for applications EP13862466, EP18190686, and EP19216609	09/12/2023	/DJS/
C12N9/6408, C12Y304/21084 with keyword search, see SEARCH printout	09/12/2023	/DJS/

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<i>Search Notes</i> 	Application/Control No. 18/346,420	Applicant(s)/Patent Under Reexamination MIZUMURA et al.
	Examiner DAVID J STEADMAN	Art Unit 1656

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

EX1002 - Page 222

SUBMISSION OF CORRECTED FORM PTO/SB/08
Appln. No.: Not Yet Assigned

Attorney Docket No.: Q289014

Cite No. 80

Indication of "Cited in cite 89" in column "T" is corrected to be "Cited in cite 84 (ISR)".

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/markboland/

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Mark Boland
Registration No. 32,197

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: August 18, 2023

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	OPAP
	Examiner Name	CENTRAL, DOCKET
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵
	20.	2012/118226	WO	A1	2012-09-07	SEIKAGAKU CORPORATION		Corresponds to cite 11 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	23.	2006-87435	JP	A	2006-04-06	SEIKAGAKU KOGYO CO LTD		English Abstract; Corresponds to cites 1 and 4 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	24.	94673	SG	A1	2003-03-18	NATIONAL UNIVERSITY OF SINGAPORE		Cited in cite 84 (ISR) in the July 3, 2023 IDS
	27.	2008/004674	WO	A1	2008-01-10	SEIKAGAKU CORPORATION		English Abstract; Corresponds to cite 13 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	29.	2005-500520	JP	A	2005-01-06	CAMBREX BIO SCIENCE WALKERVILLE INC		Corresponds to cites 14 and 18 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS

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[Page 1 of 2]

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NON-PATENT LITERATURE DOCUMENTS			
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	80.	XIANGJUN et al., "Role of domains in factor C from Tachypleus tridentatus in neutralization endotoxin", "Accession:2006:239467" File CAPLUS, [online], Retrieved from the STN on the Web, [retrieved on 2014-01-29]; Shengwu Huaxue Yu Shengwu Wuli Jinzhan, Vol. 31, No. 8, pp. 736-740, 2004	Cited in cite 84 (ISR) in the July 3, 2023 IDS

EXAMINER SIGNATURE			
Examiner Signature	/David Steadman/	Date Considered	09/11/2023

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[Page 2 of 2]

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U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	1.	6733997	B1	2004-05-11	Ding et al.	

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

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NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	2.	Extended European Search Report issued July 4, 2023 in European Application No. 22215393.4	English
	3.	Decision dated October 26, 2022 issued in European Application No. 18190686.8	English
	4.	Neus FERRER-MIRALLES et al., "General Introduction: Recombinant Protein Production and Purification of Insoluble Proteins", Methods and Protocols, Methods in Molecular Biology, 2015, Vol. 1258, pp. 1-24 (24 pages total)	English
	5.	Opponent's Reply to the Grounds of Appeal filed by the Proprietor dated July 12, 2023 filed in corresponding European Patent No. 3 441 466	English

EXAMINER SIGNATURE			
Examiner Signature	/David Steadman/	Date Considered	09/11/2023

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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	OPAP
	Examiner Name	CENTRAL, DOCKET
	Attorney Docket Number	Q289014

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

- ☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

- ☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).
- ☐ See attached certification statement.
- ☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
- ☒ The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.
- ☒ A certification statement is not submitted herewith.

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23373

CUSTOMER NUMBER

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/markboland/	Date (YYYY-MM-DD)	2023-08-18
Name/Print	Mark Boland	Registration Number	32,197

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[Page 2 of 2]

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	Confirmation Number	Not Yet Assigned
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	1.	6077946	A	2000-01-20	Iwanaga et al.	
	2.	10144923	B2	2018-12-04	Mizumura et al.	
	3.	5716834	A	1998-02-10	Ding et al.	
	4.	5795962	A	1998-08-18	Iwanaga et al.	
	5.	5840510	A	1998-11-24	Tanaka et al.	
	6.	6645724	B1	2003-11-11	Ding et al.	
	7.	5858706	A	1999-01-12	Ding et al.	
	8.	5712144	A	1998-01-27	Ding et al.	
	9.	10982202	B2	2021-04-20	Mizumura et al.	
	10.	11236318	B2	2022-02-01	Mizumura et al.	

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	11.	20140024062	A1	2014-01-23	Mizumura et al.	
	12.	20130295613	A1	2013-11-07	Kishishita et al.	
	13.	20090208995	A1	2009-08-20	Tamura et al.	
	14.	20040235080	A1	2004-11-25	Chen et al.	
	15.	20020068325	A1	2002-06-06	Ng et al.	
	16.	20100086967	A1	2010-04-08	Kaufmann et al.	
	17.	20140249082	A1	2014-09-04	Cottingham et al.	
	18.	20030054432	A1	2003-03-20	Chen et al.	
	19.	20080131929	A1	2008-06-05	Prentice et al.	

FOREIGN PATENT DOCUMENTS								
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	20.	2012/118226	WO	A1	2012-09-07	SEIKAGAKU CORPORATION		Corresponds to cite 19. Cited in cite 89
	21.	2008-520250	JP	A	2008-06-19	BIOGEN IDEC MA INC.,		Cited in JPOA 8/7/18 in no. 2014-552048; Corresponds to US 2008/0131929 A1

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[Page 1 of 6]

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

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Confirmation Number	Not Yet Assigned
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Examiner Name	DAVID J STEADMAN (Parent)
Attorney Docket Number	Q289014

FOREIGN PATENT DOCUMENTS

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	22.	9915676	WO	A1	1999-04-01	National University of Singapore		
	23.	2006-87435	JP	A	2006-04-06	SEIKAGAKU KOGYO CO LTD		English Abstract; Corresponds to cites 1 and 7; Cited in cite 89
	24.	04673	SG	A1	2003-03-18	NATIONAL UNIVERSITY OF SINGAPORE		Cited in cite 89
	25.	2012091124	WO	A1	2012-07-05	CHUGAI SEIYAKU KABUSHIKI KAISHA		Abstract; Corresponds to US 20130295613; Cited in JP comm. dated 8/7/18 in 2014-552048
	26.	2008520250	JP	A	2008-06-19	BIOGEN IDEC MA INC.		Corresponds to US 20080131929; Cited in JP comm. dated 8/7/18 in 2014-552048
	27.	2008/004674	WO	A1	2008-01-10	SEIKAGAKU CORPORATION		English Abstract; Corresponds to cite 3; Cited in cite 89
	28.	2010-516264	JP	A	2010-05-20	BOEHRINGER INGELHEIM PHARMA GMBH & CO. KG,		Cited in JPOA 8/7/18 in no. 2014-552048; Corresponds to US 2010/0086967 A1
	29.	2005-500520	JP	A	2005-04-06	CAMBREX BIO SCIENCE WALKERVILLE INC		Corresponds to cites 14 and 18; Cited in cite 89

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	30.	A certified translation of the priority document JP 2012269840 filed December 10, 2012	
	31.	Excerpt from Essential Cell Biology (2nd Edition), Alberts et al., Taylor and Francis Group, New York and London, 2004 (page 209)	
	32.	Harada-Suzuki, T. et al., "Further Studies on the Chromogenic Substrate Assay Method for Bacterial Endotoxins Using Horseshoe Crab (Tachypleus tridentatus) Hemocyte Lysate", J. Biochem., 1982, 92(3): 793-800	

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	33.	Jeak Ling Ding et al., "Chapter 9, Endotoxin Detection - from Limulus Amebocyte Lysate to Recombinant Factor C", Endotoxins: Structure, Function and Recognition SERIES : SUBCELLULAR BIOCHEMISTRY IN: SUBCELLULAR BIOCHEMISTRY: REVIEWS AND ESSAYS DEALING WITH THE FUNCTION, GENETICS, BIOGENESIS AND EVOLUTION OF SUB-CELLULAR COMPONENTS, vol. 53, January 1, 2010, XP009510157, 22 pages total, pp. 187-208	
	34.	Nakamura T. et al. "Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in Limulus hemocytes" European Journal of Biochemistry vol. 154, 1986; pages 511-521	
	35.	Office Action dated February 12, 2020 issued in US Patent Application No. 15/983,725	
	36.	The Wikipedia site relating to "Horseshoe Crab" as of Nov. 23, 2011, provided by the WayBack Machine	
	37.	Declaration by Dr. Hikaru Mizumura submitted to the USPTO, signed Feb. 22, 2018 in US14/650767	
	38.	Slade et al., "Identifying the CHO Secretome using Mucin-type O-Linked Glycosylation and Click-chemistry", J. Prot. Res., 11:6175-6186, 2012	
	39.	Bram Blomme et al., "Alteration of protein glycosylation in liver diseases", Journal of Hepatology, 2009, Vol. 50, pages 592-603 (12 pages total)	
	40.	Extended European Search Report dated January 10, 2019 issued by the European Patent Office in application no. 18190686.8	
	41.	Hossler, P. et al., "Optimal and consistent protein glycosylation in mammalian cell culture", Glycobiol. 19(9): 936-949, 2009	
	42.	JING WANG et al., "Functional expression of full length Limulus Factor C in stably transformed Sf9 cells", Biotechnology Letters, 2001, pp. 71-76, Vol. 23	
	43.	Nettleship, Joanne E., "Structural Biology of Glycoproteins", Glycosylation, 2012, p. 41-62. Available from: https://www.intechopen.com/books/glycosylation/structural-biologyof-glycoproteins ;	
	44.	Stephanie Holst et al., "Profiling of different pancreatic cancer cells used as models for metastatic behaviour shows large variation in their N-glycosylation" Scientific Reports, November 30, 2017, Vol. 7, No. 16623, Pages 1-15 (15 pages total)	
	45.	Tomiya, N. et al., "Comparing N-glycan processing in mammalian cell lines to native and engineered lepidopteran insect cell lines", Glycoconjugate Journal, 2004, 21:343-360	
	46.	Office Action dated April 27, 2020 issued in US Patent Application No. 16/399,575	
	47.	Mizumura, et al., Genetic engineering approach to develop next-generation reagents for endotoxin quantification; Innate Immunity, vol. 23, no. 2, pp. 136-146, 2017	
	48.	Damasceno et al., "Protein secretion in Pichia pastoris and advances in protein production", Appl Microbiol Biotechnol (2012) 93: 31-39, Published online: November 2011	
	49.	Office Action issued in the corresponding European Application No. 13862466.3 dated November 30, 2017	
	50.	Tokunaga F. et al. "Lipopolysaccharide-sensitive serine-protease zymogen factor C of horseshoe crab hemocytes identification and alignment of proteolytic fragments produced during the activation show that it is a novel type of serine protease" European Journal of Biochemistry vol. 167 no. 3, 1987; pages 405-416	
	51.	Sequence alignment results for Tachypleus tridentatus vs. Limulus polyphemus, cited in Submission from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	52.	Roche, "DIG Glycan Differentiation Kit Instructions", 2018, Cat. No. 11 210 238 001, pp. 1-5 (5 pages total)	
	53.	Snapshot of the Wikipedia article for "Intravenous sodium bicarbonate (sodium hydrogencarbonate)", as of April 25, 2013 (3 pages total)	
	54.	Su Chen et al., "Production of Recombinant Proteins in Mammalian Cells", Current Protocols in Protein Science, 1998, pp. 5.10.1-5.10.41 (41 pages total)	

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³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 3 of 6]

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /DJS/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	Not Yet Assigned
	Confirmation Number	Not Yet Assigned
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	55.	"SignalP results for Carinoscorpius rotundicauda", cited in Submission from the Opponent dated September 1, 2022 in European Patent No. 3 441 466	
	56.	"SignalP results for Tachypleus tridentatus", cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	57.	Takao Hashiguchi et al., "Expression of Recombinant Proteins in Cultured Human Cells Standard Protocol with 293 strain cells", Protein Science Society Archive, 2008, 1, e017, pp. 1-9 (18 pages total)	Machine Translation; cited in Submission from the opponent dated 9/1/22 in EP Patent No. 3 441 466
	58.	"FAQ-Why do I see too many bands on my Western blot", available from: https://sysy.com/resources/faq/too-many-bands-on-a-western-blot , Synaptic Systems, cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	59.	Communication dated August 10, 2022 regarding the submission filed by the opponent in the EP Patent No. 3441466 on August 4, 2022	
	60.	Merriam-Webster entry for the term "intracellular", cited in Submissions from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	61.	Ina Kraus, "Das Matrixprotein des Virus der Bornaschen Krankheit (The matrix protein of the virus of Borna disease)", 2002 (132 pages total)	Partial Translation; cited in Submission of the opponent dated 8/4/22 in EP Patent No. 3441466
	62.	"Overview of Cell Lysis and Protein Extraction", ThermoFisher Scientific, available from https://www.thermofisher.com/de/de/home/life-science/protein-biology/protein-biologylearning-center/protein-biology-resource-library/pierce-protein-methods/overview-celllysis-and-protein-extraction.html , cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466 (7 pages total)	
	63.	Khar Heng Choo et al., "A Comprehensive assessment of N-terminal signal peptides prediction methods", BMC Bioinformatics, 2009, 10(Suppl 15):S2, pp.1-12 (12 pages total)	
	64.	NCBI Database entry for the Factor C sequence derived from Carinoscorpius rotundicauda, NCBI, retrieved August 23, 2022 from https://www.ncbi.nlm.nih.gov/protein/AAB34361.1 , GenBank: AAB34361.1 (2 pages total)	
	65.	Invitrogen, "FreeStyle 293 Expression System", User Manual, 2010, 23 pages total	
	66.	"Troubleshooting Tips-WB", ABCAM, 2011 (3 pages total)	
	67.	Submission filed by the opponent in the EP Patent No. 3441466 dated September 1, 2022	
	68.	Decision of the Technical Board of Appeal dated May 3, 2000 issued in European Application No. 83301269.3	
	69.	Submission dated April 5, 2018 in the European Application No. 13 862 466.3	
	70.	David Gray, "Overview of Protein Expression by Mammalian Cells", Current Protocols in Protein Science, 1997, pp. 5.9.1-5.9.18 (18 pages total)	
	71.	Henrik Nielsen et al., "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites", Protein Engineering, 1997, Vol. 10, No.1, pp.1-6 (6 pages total)	

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[Page 4 of 6]

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	Not Yet Assigned
Confirmation Number	Not Yet Assigned
Filing Date	July 3, 2023
First Named Inventor	Hikaru MIZUMURA
Art Unit	1656 (Parent)
Examiner Name	DAVID J STEADMAN (Parent)
Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	72.	Jeak L. Ding et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, <i>Carcinoscorpius rotundicauda</i> ", Molecular Marine Biology and Biotechnology, 1995, Vol. 4, No. 1, pp. 90-103 (14 pages total)	
	73.	Invitrogen, "FreeStyle Max Reagent", User Guide, 2013, pp.1-3 (3 pages total)	
	74.	Claire Moore, "Introduction to Western Blotting", 2009, pp. 1-48 (26 pages total)	
	75.	Shengwu Wuli Jinzhan Vol.31, No.8, p736-740, 2004	
	76.	Communication dated April 19, 2017, from the State Intellectual Property Office of the P.R.C., in Chinese application no. 201380064649.3	Translation
	77.	Final Office Action dated August 4, 2020 issued in U.S. Application 16/399,575	
	78.	JEAK L. DING et al., "A new era in pyrogen testing", TRENDS in Biotechnology, August 2001, pp. 277-281, Vol. 19, No. 8	
	79.	Knappskog et al., "The level of synthesis and secretion of <i>Ganssia princeps</i> luciferase in transfected CHO cells is heavily dependent on the choice of signal peptide", Journal of Biotechnology, Vol. 128, 2007, 705-715	
	80.	XIANGJUN et al., "Role of domains in factor C from <i>Tachypleus tridentatus</i> in neutralization endotoxin", Accession: 2006-239467 File CAPLUS, [online], Retrieved from the STN on the Web, [retrieved on 2014-01-29]; Shengwu Huaxue Yu Shengwu Wuli Jinzhan, Vol. 31, No. 8, pp. 736-740, 2004	Cited in cite 89
	81.	The Wikipedia site relating to "Blood Plasma" as of Nov. 19, 2012, provided by the WayBack Machine	
	82.	Declaration by Dr. Hikaru Mizumura submitted to the USPTO, signed Oct. 11, 2017 in US14/650767	
	83.	Koshiha et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria", J. Biol. Chem., 282:3962-3967, 2007	
	84.	International Search Report for PCT/JP2013/083082 dated February 10, 2014	English Translation
	85.	Communication dated July 5, 2016, from the European Patent Office in European Application No. 13862466.3	
	86.	TAKANORI NAKAMURA et al., "Purification and Properties of Intracellular Clotting Factor, Factor B, from Horseshoe Crab (<i>Tachypleus tridentatus</i>) Hemocytes", J. Biochem, 1986, Vol. 99, No. 3, pp. 847-857	
	87.	International Preliminary Report on Patentability in International Application No. PCT/JP2013/083082 issued June 16, 2015 and mailed June 25, 2015	
	88.	Carlesso, E. et al., "The rule regulating pH changes during crystalloid infusion", Intensive Care Med., 2011, 37(3): 461-468	
	89.	Final Office Action dated August 28, 2020 issued in U.S. Application 15/983,725	
	90.	Inhibition data performed using a Limulus Factor C expressed in HEK 293 cells and HEK 293 GnTI- cells filed with Opposition against EP3441466B dated November 5, 2020	
	91.	JING WANG et al., "Modular Arrangement and Secretion of a Multidomain Serine Protease", The Journal of Biological Chemistry, September 27, 2002, pp. 36363-36372, Vol. 277, No. 39	
	92.	Office Action dated May 21, 2020 issued in U.S. Application No. 15/983,725	
	93.	Communication of Notice of Opposition dated November 11, 2020, issued by the European Patent Office in EP Application no. 18190686.8	
	94.	Thomas, P. and Smart, T.G., "HEK293 cell line: A vehicle for the expression of recombinant proteins", J. Pharmacol. And Toxicol. Methods, 2005, 51: 187-200	
	95.	Strategies for Protein Purification Handbook, GE Healthcare, September 2010, 167 pages	
	96.	Written Opinion for PCT/JP2013/083082 dated February 10, 2014	

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[Page 5 of 6]

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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	97.	Communication dated July 29, 2016 from the State Intellectual Property Office of the P.R.C. in counterpart application no. 201380064649.3	
	98.	SADAAKI IWANAGA, "The limulus clotting reaction", Current Opinion in Immunology 1993, pp. 74-82, Vol. 5	
	99.	Current Protocols in Molecular Biology (1993) 16.23.1-16.23.13, John Wiley and Sons, Inc., 2002	
	100.	Communication dated August 7, 2018 issued by the Japanese Patent Office in application no. 2014-552048	Machine Translation
	101.	ROOPASHREE S. DWARAKANATH et al., "Recombinant COS-1 cells express Carcinoscopus rotundicauda Factor C", Biotechnology Letters, April 1997, pp. 357-361, Vol. 19, No. 4	
	102.	Merriam-Webster entry for the term "insoluble", cited in Submission from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	103.	Snapshot of the Wiktionary entry for "+ve" as of October 21, 2012 (1 page total)	
	104.	PubChem entry for "sodium bicarbonate (sodium hydrogencarbonate)", 2022 (1 page total)	
	105.	The Wikipedia entry for "Signal peptide", as of November 23, 2012 (3 pages total)	
	106.	SignalP 6.0, "Signal peptides and their cleavage sites in all domains of life", 2022 (1 page total)	
	107.	"SignalP results for Limulus polyphemus", cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	108.	"Troubleshooting Western Blotting", Hycult biotech, 2010 (3 pages total)	
	109.	Third Party Observations dated October 10, 2022 submitted in European Application No. 19216609.8 Forwarded by EPO on October 13, 2022	
	110.	XIANGJUN et al., "Role of domains in factor C from Tachypleus tridentatus in neutralization endotoxin", "Accession:2006:239467" File CAPLUS, 2003	

EXAMINER SIGNATURE			
Examiner Signature	/David Steadman/	Date Considered	09/11/2023

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[Page 6 of 6]

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Web Search History

date, time	web site	search string
9/12/2023 5:02:03 AM	Google	(secrete OR secretion OR excrete OR excretion) (horseshoe OR crab) factor
9/12/2023 5:02:44 AM	Google Scholar	(secrete OR secretion OR excrete OR excretion) (horseshoe OR crab) factor
9/12/2023 5:03:13 AM	Google Scholar	(secrete OR secretion OR excrete OR excretion) (horseshoe OR crab) factor [before:2013]
9/12/2023 5:04:07 AM	Google Scholar	[before:2013] (secrete OR secretion OR excrete OR excretion) recombinant (horseshoe OR crab) factor
9/12/2023 5:05:13 AM	Google Scholar	[before:2013] (secrete OR secretion OR excrete OR excretion) recombinant (horseshoe OR crab) factor CHO
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9/12/2023 5:08:02 AM	Google Scholar	[before:2013] recombinant (horseshoe OR crab) factor HEK293

Bibliographic Data

Application No: 18/346,420

Foreign Priority claimed: ☒ Yes ☐ No

35 USC 119 (a-d) conditions met: ☒ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged:

/David Steadman/

Examiner's Signature

Initials

Title:

NOVEL RECOMBINANT FACTOR C AND METHOD FOR
PRODUCING THE SAME, AND METHOD FOR MEASURING
ENDOTOXIN

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/03/2023	435	1656	Q289014
RULE			

APPLICANTS

Seikagaku Corporation, Tokyo, JAPAN

INVENTORS

Hikaru MIZUMURA,

Toshio Oda,

Shun-Ichiro Kawabata,

CONTINUING DATA

This application is a CON of 17554128 12/17/2021

17554128 is a CON of 16399386 04/30/2019 PAT 11236318

16399386 is a CON of 15983725 05/18/2018 PAT 10982202

15983725 is a CON of 14650767 06/09/2015 PAT 10144923

14650767 is a 371 of PCT/JP2013/083082 12/10/2013

FOREIGN APPLICATIONS

JAPAN JP2012-269840 12/10/2012

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07/27/2023

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PE2E SEARCH - Search History (Prior Art)

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			JPO; DERWENT)				
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L55	1	L54 not L53	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 11:49 AM
L56	2	"9133495".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:36 PM
L57	19132	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L58	6	L57 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L59	2485	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L60	3	L59 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L61	7	L58 or L60	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2017/10/31 03:45 PM
L62	19414	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L63	6	L62 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L64	2493	seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L65	3	L64 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L66	7	L63 or L65	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/04/12 03:58 PM
L67	19635	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L68	6	L67 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/08/14 09:19 AM
L80	3	("20080131929" "20100086967" "20130295613").PN.	(US-PGPUB; USPAT)	OR	OFF	OFF	2018/09/07 10:58 AM
L81	19653	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM

			JPO; DERWENT)				
L82	6	L81 AND (dg44 OR dg-44 OR chodg44 OR cho-dg44)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2018/09/07 11:43 AM
L94	6	"10144923".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 09:23 AM
L96	6	(US-20080131929-\$ US-20100086967-\$ US-20130295613-\$).DID.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/01 10:53 AM
L97	20399	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L98	40	L97 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2019/10/02 03:15 PM
L99	23093	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:15 AM
L100	95	L99 AND (tridentatus OR polyphemus OR rotundicauda OR gigas)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L101	54	L100 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L102	24	L101 and ("chinese hamster" OR CHO OR human OR HEK)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/02/07 10:16 AM
L103	627	tridentatus OR rotundicauda	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L104	389	L103 and endotoxin	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L105	263	L104 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L106	224	L103 same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L107	216	L105 and L106	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:41 PM
L108	140	L107 and "37"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L109	1050017	"2 hours"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM
L110	114	L108 and L109	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:42 PM

			USOCR; FPRS; EPO; JPO; DERWENT)				02:42 PM
L113	349173	cascade	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:44 PM
L114	169	L107 and L113	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 02:45 PM
L115	468	limulus adj test	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:30 PM
L116	1	L115 same cation	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/20 03:31 PM
L117	3	"6,645,724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 12:19 PM
L118	13	("20030054432" "20040 235080" "20080131929 " "20090208995" "2010 0086967" "2013029561 3" "20140024062" "201 40249082" "5712144" "5 716834" "5795962" "5 858706" "6077946").PN .	(US-PGPUB; USPAT)	OR	OFF	OFF	2020/04/22 02:32 PM
L119	23218	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L120	85	L119 AND (tridentatus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:37 PM
L121	54	L120 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:38 PM
L122	33	L121 and endotoxin.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/04/22 02:39 PM
L123	23351	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L124	95	L123 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:10 PM
L125	55	L124 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L126	10	L125 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:11 PM
L127	8	L125 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2020/06/24 03:12 PM

			JPO; DERWENT)				
L128	10	L126 or L127	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/06/24 03:12 PM
L129	23458	(mizumura-h\$ OR oda- t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L130	98	L129 AND (tridentatus OR rotundicauda OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L131	57	L130 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L132	11	L131 and (cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L133	9	L131 and (hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L134	11	L132 or L133	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:30 AM
L135	753	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:52 AM
L136	44	L135 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L137	829430	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L138	104016	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L139	14	L136 and (L137 OR L138)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2020/10/02 11:53 AM
L140	4	"6645724".pn.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L141	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L142	46	L141 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L143	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM
L144	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 04:49 AM

			JPO; DERWENT)				
L145	787	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L146	46	L145 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L147	854970	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L148	107501	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L149	15	L146 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:20 AM
L150	64	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L151	24	L150 and (L147 OR L148)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/03/04 06:26 AM
L152	886720	(cho OR "chinese hamster")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L153	112690	(hek OR (human adj embryo\$))	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L154	69	C12Y304/21084.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L155	19	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 07:59 AM
L156	812	c12n9/6408.cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L157	50	L156 and (tridentatus OR polyphemus OR rotundicauda)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L158	16	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:00 AM
L159	40	L156 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:30 AM
L160	13	(L152 OR L153) same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:31 AM
L161	204	(L152 OR L153) same "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	OFF	2021/10/18 08:40 AM

			JPO; DERWENT)				
L162	13	L160 and (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:41 AM
L163	4875	(hek OR hek293 OR (human adj embryo\$)).clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L164	8	L157 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:48 AM
L165	14	L154 and hek293	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L166	6	L165 not L164	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:49 AM
L167	8	hek293 same (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:50 AM
L168	24311	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L169	103	L168 AND (tridentatus OR polyphemus)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L170	63	L169 and "factor c"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:51 AM
L171	15	L170 and (L152 or L153 OR hek293)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2021/10/18 08:52 AM
L172	2183	ding-J\$.in.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L173	22336	"factor c"	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:55 PM
L174	26	172 AND 173	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L175	722713	secret\$	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L176	13	174 AND 175	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L177	563	tachypleus	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/13 03:56 PM
L178	172826	ding-J\$.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2023/03/13 03:56 PM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L179	81001	"factor c"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:56 PM
L180	117	178 AND 179	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L181	2156	tachypleus	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM

L182	17	180 AND 181	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L183	3284055	secret\$	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L184	13	182 AND 183	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/13 03:57 PM
L185	93965	"factor b"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO,	OR	ON	ON	2023/03/14 09:54 AM

			MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L188	438	185 AND 177	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:55 AM
L189	3617370	"chinese hamster" OR CHO????	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM
L190	104	188 AND 189	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/14 09:56 AM
L191	21	recombinant?? adj5 185	(US-PGPUB; USPAT;	OR	ON	ON	2023/03/14

			USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				10:04 AM
L192	12	("0000001" OR "10144923" OR "20020068325" OR "20030054432" OR "20040235080" OR "20080131929" OR "20090208995" OR "20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5795962").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/03/14 05:22 PM
L193	21	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 05:59 PM
L194	115	L156 AND (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L195	10351	limulus OR tachypleus	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:01 PM
L196	25	194 AND 195	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L197	9	196 NOT 193	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/14 06:02 PM
L198	2271	"factor c".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:49 AM
L199	25141	(mizumura-h\$ OR oda-t\$ OR kawabata-s\$).in. OR seikagaku.as.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L200	27	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/03/15 05:50 AM
L201	379035	(human ADJ embryo\$) OR hek???	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY,	OR	ON	ON	2023/03/15 05:51 AM

			CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L202	3817922	189 OR 201	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:51 AM
L203	37	200 AND 202	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/03/15 05:52 AM
L204	28	198 AND 199	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/11 03:26 PM
L205	19	("10144923" OR "10982202" OR "11236318" OR "20020068325" OR "20030054432" OR "20040235080" OR "20080131929" OR "20090208995" OR	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/09/11 04:10 PM

		"20100086967" OR "20130295613" OR "20140024062" OR "20140249082" OR "5712144" OR "5716834" OR "5795962" OR "5840510" OR "5858706" OR "6077946" OR "6645724").pn.					
L206	1	("6733997").pn.	(US-PGPUB; USPAT)	OR	OFF	OFF	2023/09/11 04:10 PM
L207	22	L154 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:10 AM
L208	24	L157 and (L152 OR L153)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM
L209	29	207 OR 208	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT)	OR	OFF	OFF	2023/09/12 05:12 AM

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E6	10	MIZUMURA HIROMI/AU
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E20	1	MIZUMURA KAZUMASA/AU
E21	14	MIZUMURA KAZUO/AU

E22	1	MIZUMURA KEIICHI/AU
E23	1	MIZUMURA KEISUKE/AU
E24	39	MIZUMURA KENJI/AU
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L1 26 "MIZUMURA HIKARU"/AU

=> E oda toshio/AU 25

E1	9	ODA TOSHINARI/AU
E2	4	ODA TOSHINORI/AU
E3	94 -->	ODA TOSHIO/AU
E4	55	ODA TOSHIRO/AU
E5	1	ODA TOSHIROU/AU
E6	3	ODA TOSHISATO/AU
E7	2	ODA TOSHITADA/AU
E8	2	ODA TOSHITAKE/AU
E9	181	ODA TOSHITSUGU/AU
E10	5	ODA TOSHITUGU/AU
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E13	1	ODA TOSHUKI/AU
E14	1	ODA TOSIO/AU
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E17	3	ODA TOYOHIRO/AU
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E20	1	ODA TSUENO/AU
E21	11	ODA TSUGIO/AU
E22	2	ODA TSUGUMI/AU
E23	46	ODA TSUKASA/AU
E24	4	ODA TSUNEAKI/AU
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L2 94 "ODA TOSHIO"/AU

=> E kawabata shun/AU 25

E1	7	KAWABATA SHUJI/AU
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E3	10 -->	KAWABATA SHUN/AU
E4	144	KAWABATA SHUN ICHIRO/AU
E5	6	KAWABATA SHUNGO/AU
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E7	62	KAWABATA SHUNICHIRO/AU
E8	1	KAWABATA SHUNICHIROU/AU
E9	5	KAWABATA SHUNJI/AU
E10	1	KAWABATA SHUNSAKU/AU
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E13	6	KAWABATA SHUSAKU/AU
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E17	1	KAWABATA SKIRA/AU
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E19	1	KAWABATA SORA/AU
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=> s e4

L3 144 "KAWABATA SHUN ICHIRO"/AU

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E MIZUMURA HIKARU/AU 25

L1 26 S E3
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L2 94 S E3
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L3 144 S E4

=> s l1 or l2 or l3

L4 247 L1 OR L2 OR L3

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L8 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2018:2358618 CAPLUS Full-text

DOCUMENT NUMBER: 182:391398

TITLE: Pluripotency and a secretion mechanism of Drosophila
transglutaminase

AUTHOR(S): Shibata, Toshio; Kawabata, Shun-ichiro

CORPORATE SOURCE: Institute for Advanced Study, Kyushu University,
Fukuoka, 819-0395, Japan

SOURCE: Journal of Biochemistry (2018), 163(3), 165-176

CODEN: JOBIAO; ISSN: 1756-2651

DIGITAL OBJECT ID: 10.1093/jb/mvx059

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal; General Review; (online computer file)

LANGUAGE: English

AB A review. Transglutaminase (TG) catalyzes the formation of an isopeptide bond between glutamine and lysine residues and amine incorporation into specific glutamine residues. TG is conserved in all metazoans and functions both intracellularly and extracellularly. Here we review the existing knowledge of Drosophila TG with an emphasis on its pluripotency: Drosophila TG (i) plays a key role in cuticular morphogenesis, haemolymph coagulation, and entrapment against invading pathogens, (ii) suppresses the immune deficiency pathway to enable immune tolerance against commensal bacteria through the incorporation of polyamines into the nuclear factor- κ B-like transcription factor Relish as well as through

the protein-protein crosslinking of Relish, (iii) forms a phys. matrix in the gut through crosslinking of chitin-binding proteins and (iv) is involved in the maintenance of homeostasis in microbiota in the gut. Moreover, we review the evidence that TG-A, one of alternative splicing-derived isoforms of *Drosophila* TG, is ~~secreted~~ through an endoplasmic reticulum/Golgi-independent pathway involving exosomes and fatty acylations.

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L8 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2012:1025087 CAPLUS Full-text
 DOCUMENT NUMBER: 158:269439
 TITLE: Immunocompetent molecules and their response network in horseshoe crabs
 AUTHOR(S): Kawabata, Shun-ichiro
 CORPORATE SOURCE: Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka, Japan
 SOURCE: Advances in Experimental Medicine and Biology (2010), 708(Invertebrate Immunity), 122-136
 CODEN: AEMBAP; ISSN: 0065-2598
 PUBLISHER: Springer
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: English

AB A review. Horseshoe crab hemocyte selectively responds to bacterial lipopolysaccharides (LPS), which depends critically on the proteolytic activity of the LPS-responsive serine protease zymogen factor C. In response to stimulation by LPS, the hemocyte ~~secreted~~ several kinds of immunocompetent proteins. The coagulation cascade triggered by LPS or β -1,3-D-glucans (BDG) results in the formation of coagulin fibrils that are subsequently stabilized by transglutaminase (TGase)-dependent crosslinking. Invading pathogens are recognized and agglutinated by lectins and then killed by antimicrobial peptides. Moreover, LPS-triggered hemocyte exocytosis is enhanced by a feedback mechanism in which the antimicrobial peptides serve as endogenous mediators. Factor C also acts as an LPS-sensitive complement C3 convertase. In addn., a sub-cuticular epidermis-derived protein forms a TGase-stabilized mesh at sites of injury. Horseshoe crabs have a sophisticated innate immune response network that coordinately effects pathogen recognition and killing, prophenoloxidase activation, complement activation and TGase-dependent wound healing.

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L8 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2012:762058 CAPLUS Full-text
 DOCUMENT NUMBER: 159:24755
 TITLE: Microbe-specific C3b deposition in the horseshoe crab complement system in a C2/factor B-dependent or -independent manner
 AUTHOR(S): Tagawa, Keisuke; Yoshihara, Toyoki; Shibata, Toshio; Kitazaki, Kazuki; Endo, Yuichi; Fujita, Teizo; Koshiba, Takumi; Kawabata, Shun-ichiro
 CORPORATE SOURCE: Graduate School of Systems Life Sciences, Kyushu University, Fukuoka, Japan
 SOURCE: PLoS One (2012), 7(5), e36783
 CODEN: POLNCL; ISSN: 1932-6203
 DIGITAL OBJECT ID: 10.1371/journal.pone.0036783
 PUBLISHER: Public Library of Science
 DOCUMENT TYPE: Journal; (online computer file)
 LANGUAGE: English

AB Complement C3 plays an essential role in the opsonization of pathogens in the mammalian complement system, whereas the mol. mechanism underlying C3 activation in invertebrates remains unknown. To understand the mol. mechanism of C3b deposition on microbes, we characterized two types of C2/factor B homologs (designated TtC2/Bf-1 and TtC2/Bf-2) identified from the horseshoe crab *Tachypleus tridentatus*. Although the domain architectures of TtC2/Bf-1 and TtC2/Bf-2 were identical to those of

mammalian homologs, they contained five-repeated and seven-repeated complement control protein domains at their N-terminal regions, resp. TtC2/Bf-1 and TtC2/Bf-2 were synthesized and glycosylated in hemocytes and secreted to hemolymph plasma, which existed in a complex with C3 (TtC3), and their activation by microbes was absolutely Mg²⁺-dependent. Flow cytometric anal. revealed that TtC3b deposition was Mg²⁺-dependent on Gram-pos. bacteria or fungi, but not on Gram-neg. bacteria. Moreover, this anal. demonstrated that Ca²⁺-dependent lectins (C-reactive protein-1 and tachylectin-5A) were required for TtC3b deposition on Gram-pos. bacteria, and that a Ca²⁺-independent lectin (Tachypleus plasma lectin-1) was definitely indispensable for TtC3b deposition on fungi. In contrast, a horseshoe crab lipopolysaccharide-sensitive protease factor C was necessary and sufficient to deposit TtC3b on Gram-neg. bacteria. We conclude that plasma lectins and factor C play key roles in microbe-specific TtC3b deposition in a C2/factor B-dependent or -independent manner.

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L8 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2012:599120 CAPLUS Full-text

DOCUMENT NUMBER: 157:41243

TITLE: Innate immune response and regulation against pathogens in horseshoe crab

AUTHOR(S): Shibata, Toshio; Kawabata, Shun-ichiro

CORPORATE SOURCE: Grad. Sch. Sci., Kyushu University, Japan

SOURCE: Kagaku to Seibutsu (2012), 50(4), 277-282

CODEN: KASEAA; ISSN: 0453-073X

PUBLISHER: Gakkai Shuppan Senta

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese

AB A review on recognition of infectious pathogen recognition, enclosure, disinfect, removal, and wound healing as innate immune responses in horseshoe crab; hemocyte exocytotic degranulation and secretion of various defense mols. such as coagulation factors, antimicrobial peptides, and lectins induced by LPS, existence of TLR in horseshoe crab, G factor and C factor in hemolymph coagulation, multifunction of antimicrobial peptides, and transglutaminase induction of coagulation and wound healing.

L8 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2006:682218 CAPLUS Full-text

DOCUMENT NUMBER: 145:164844

TITLE: Recognition of pathogens and activation of immune responses in Drosophila and horseshoe crab innate immunity

AUTHOR(S): Kurata, Shoichiro; Ariki, Shigeru; Kawabata, Shun-ichiro

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, 980-8578, Japan

SOURCE: Immunobiology (2006), 211(4), 237-249

CODEN: IMMND4; ISSN: 0171-2985

DIGITAL OBJECT ID: 10.1016/j.imbio.2005.10.016

PUBLISHER: Elsevier GmbH

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review. In innate immunity, pattern recognition receptors discriminate between self- and infectious non-self-matter. Mammalian homologs of the Drosophila Toll protein, which are collectively referred to as Toll-like receptors (TLRs), recognize pathogen-assocd. mol. patterns (PAMPs), including lipopolysaccharides (LPS) and lipoproteins, whereas the Drosophila Toll protein does not act as a PAMP receptor, but rather binds to Spatzle, an endogenous peptide. In Drosophila, innate immune surveillance is mediated by members of the peptidoglycan recognition protein (PGRP) family, which recognize diverse bacteria-derived peptidoglycans and initiate appropriate immune reactions including the release of antimicrobial peptides and the activation of the prophenoloxidase cascade, the latter effecting localized wound healing, melanization, and microbial phagocytosis. In the horseshoe crab, LPS induces hemocyte exocytotic degranulation, resulting in the secretion of various defense mols., such as coagulation factors, antimicrobial

peptides, and lectins. Recent studies have demonstrated that the zymogen form of the serine protease factor C, a major granular component of hemocyte, also exists on the hemocyte surface and functions as a biosensor for LPS. The proteolytic activity of activated factor C initiates hemocyte exocytosis via a G protein mediated signal transduction pathway. Furthermore, it has become clear that an endogenous mechanism for the feedback amplification of the innate immune response exists and is dependent upon a granular component of the horseshoe crab hemocyte.

OS.CITING REF COUNT: 88 THERE ARE 88 CAPLUS RECORDS THAT CITE THIS RECORD (88 CITINGS)
 REFERENCE COUNT: 83 THERE ARE 83 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2004:110734 CAPLUS Full-text

DOCUMENT NUMBER: 140:232814

TITLE: A serine protease zymogen functions as a pattern-recognition receptor for lipopolysaccharides
 AUTHOR(S): Ariki, Shigeru; Koori, Kumiko; Osaki, Tsukasa; Motoyama, Kiyohito; Inamori, Kei-ichiro; Kawabata, Shun-ichiro

CORPORATE SOURCE: Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka, 812-8581, Japan

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(4), 953-958
 CODEN: PNASA6; ISSN: 0027-8424

DIGITAL OBJECT ID: 10.1073/pnas.0306904101

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Bacterial lipopolysaccharide (LPS)-induced exocytosis of granular hemocytes is a key component of the horseshoe crab's innate immunity to infectious microorganisms; stimulation by LPS induces the secretion of various defense mols. from the granular hemocytes. Using a previously uncharacterized assay for exocytosis, the authors clearly show that hemocytes respond only to LPS and not to other pathogen-assocd. mol. patterns, such as β -1,3-glucans and peptidoglycans. Furthermore, the authors show that a granular protein called factor C, an LPS-recognizing serine protease zymogen that initiates the hemolymph coagulation cascade, also exists on the hemocyte surface as a biosensor for LPS. The authors' data demonstrate that the proteolytic activity of factor C is both necessary and sufficient to trigger exocytosis through a heterotrimeric GTP-binding protein-mediating signaling pathway. Exocytosis of hemocytes was not induced by thrombin, but it was induced by hexapeptides corresponding to the tethered ligands of protease-activated G protein-coupled receptors (PARs). This finding suggested the presence of a PAR-like receptor on the hemocyte surface. The authors conclude that the serine protease zymogen on the hemocyte surface functions as a pattern-recognition protein for LPS.

OS.CITING REF COUNT: 67 THERE ARE 67 CAPLUS RECORDS THAT CITE THIS RECORD (68 CITINGS)

REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 1998:145198 CAPLUS Full-text

DOCUMENT NUMBER: 128:293660

ORIGINAL REFERENCE NO.: 128:58175a,58178a

TITLE: New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions

AUTHOR(S): Iwanaga, Sadaaki; Kawabata, Shun-ichiro; Muta, Tatsushi

CORPORATE SOURCE: Department of Biology, Faculty of Science, Kyushu University, Fukuoka, 812-81, Japan

SOURCE: Journal of Biochemistry (1998), 123(1), 1-15
 CODEN: JOBIAO; ISSN: 0021-924X

DIGITAL OBJECT ID: 10.1093/oxfordjournals.jbchem.a021894

PUBLISHER: Japanese Biochemical Society

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review, with 112 refs. Invertebrate animals, which lack adaptive immune

systems, have developed defense systems, so-called innate immunity, that respond to common antigens on the surface of potential pathogens. One such defense system is involved in the cellular responses of horseshoe crab hemocytes to invaders. Hemocytes contain 2 types, large (L) and small (S), of secretory granules, and the contents of these granules are released in response to invading microbes via exocytosis. Recent biochem. and immunol. studies on the granular components of L- and S-granules demonstrated that the 2 types of granules selectively store granule-specific proteins participating in the host defense systems. L-Granules contain all the clotting factors essential for hemolymph coagulation, protease inhibitors including serpins and cystatin, and anti-lipopolysaccharide (LPS) factor and several tachylectins with LPS binding and bacterial agglutinating activities. On the other hand, S-granules contain various new cysteine-rich basic proteins with antimicrobial or bacterial agglutinating activities, such as tachyplesins, big defensin, tachycitin, and tachystatins. The co-localization of these proteins in the granules and their release into the hemolymph suggest that they serve synergistically to construct an effective host defense system against invaders. Here, the structures and functions of these new types of defense mols. found in the Japanese horseshoe crab (*Tachypleus tridentatus*) are reviewed.

OS.CITING REF COUNT: 226 THERE ARE 226 CAPLUS RECORDS THAT CITE THIS RECORD (227 CITINGS)
 REFERENCE COUNT: 96 THERE ARE 96 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 1995:316763 CAPLUS Full-text

DOCUMENT NUMBER: 123:165369

ORIGINAL REFERENCE NO.: 123:29399a,29402a

TITLE: A limulus intracellular coagulation inhibitor type 2. Purification, characterization, cDNA cloning, and tissue localization

AUTHOR(S): Miura, Yoshiki; Kawabata, Shun-ichiro; Wakamiya, Yakako; Nakamura, Takanori; Iwanaga, Sadaaki

CORPORATE SOURCE: Dep. Biol., Kyushu University, Fukuoka, 812, Japan

SOURCE: Journal of Biological Chemistry (1995), 270(2), 558-65
 CODEN: JBCHA3; ISSN: 0021-9258

DIGITAL OBJECT ID: 10.1074/jbc.270.2.558

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We described in a foregoing report findings on serpin, a serine protease inhibitor, newly identified in horseshoe crab (*Tachypleus tridentatus*) hemocytes and we name it limulus intracellular coagulation inhibitor, LICI (Miura, Y., Kawabata, S., and Iwanaga, S. (1994) J. Biol. Chem. 269, 542-547). This serpin specifically inhibits limulus lipopolysaccharide-sensitive serine protease, factor C. In ongoing studies on limulus serpin, we have found another inhibitor, LICI type-2 (LICI-2), which inhibits not only factor C ($k_1 = 7.1 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) but also limulus clotting enzyme ($k_1 = 4.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$). LICI-2 inhibits mammalian serine proteases, including α -thrombin, salivary kallikrein, plasmin, and tissue plasminogen activator. The inactivation of plasmin is the most rapid ($k_1 = 1.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$). The purified LICI-2 is a single chain glycoprotein with an apparent $M_r = 42,000$. A cDNA for LICI-2 was isolated and the open reading frame coded for a mature protein of 386 amino acids, of which 160 residues were confirmed by peptide sequencing. Although LICI-2 shows significant sequence similarity to the previous limulus serpin, LICI-1 (42% identity), LICI-2 contains a unique putative reactive site, -Lys-Ser-, distinct from that of LICI-1 (-Arg-Ser-). Northern blotting revealed expression of LICI-2 mRNA only in hemocytes, and not in heart, brain, stomach, intestine, coxal gland, and skeletal muscle. The immunoblot of large and small granule components with antiserum against purified LICI-2 suggests that LICI-2 is stored specifically in large granules, as in the case of LICI-1, and is released in response to external stimuli. We propose that the LICIs be classified into a new subfamily of intracellular serpins, regulated secretory serpins.

OS.CITING REF COUNT: 48 THERE ARE 48 CAPLUS RECORDS THAT CITE THIS RECORD (48 CITINGS)

=> d his

(FILE 'HOME' ENTERED AT 05:42:13 ON 12 SEP 2023)

FILE 'CAPLUS' ENTERED AT 05:42:36 ON 12 SEP 2023

L1 26 S E3
 E MIZUMURA HIKARU/AU 25
L2 94 S E3
 E ODA TOSHIO/AU 25
 E KAWABATA SHUN/AU 25
L3 144 S E4
L4 247 S L1 OR L2 OR L3
L5 3193236 S FACTOR
L6 72 S L4 AND L5
L7 949971 S (SECRET? OR EXCRET?)
L8 8 S L6 AND L7

=> s l6 and recombinant

 469216 RECOMBINANT
L9 9 L6 AND RECOMBINANT

=> s l9 not l8

L10 9 L9 NOT L8

=> d ibib abs 1-

YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2021:1536297 CAPLUS Full-text
TITLE: Collaborative study on the bacterial endotoxins test
 using recombinant factor C-based procedure for
 detection of lipopolysaccharides
AUTHOR(S): Kikuchi, Yutaka; Haishima, Yuji; Fukui, Chie; Murai,
 Toshimi; Nakagawa, Yukari; Ebisawa, Akiko; Matsumura,
 Kayoko; Ouchi, Kazuyuki; Oda, Toshio; Mukai, Motoki;
 Masuda, Tamaki; Katto, Yuka; Takasuga, Yoshihiro;
 Takaoka, Aya
CORPORATE SOURCE: National Institute of Health Sciences, 1-18-1
 Kamiyoga, Setagaya-ku, Tokyo, 158-8501, Japan
SOURCE: Iyakuhin Iryo Kiki Regyuratori Saiensu (2017), 48(4),
 252-260
 CODEN: IIKRA9; ISSN: 1884-6076
PUBLISHER: Nippon Koteisho Kyokai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Unavailable
OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD
 (4 CITINGS)

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2021:1313839 CAPLUS Full-text
TITLE: Collaborative study on the bacterial endotoxins test
 using recombinant factor C-based procedure for
 detection of lipopolysaccharides (part 2)
AUTHOR(S): Kikuchi, Yutaka; Haishima, Yuji; Fukui, Chie;
 Nakagawa, Yukari; Ebisawa, akiko; Morioka, Tomoko;
 Matsumura, Kayoko; Ouchi, Kazuyuki; Uchida, Kazuyuki;
 Martinez, Olivier; Oda, Toshio; Mukai, Motoki;
 Masuda, Tamaki; Tsukihashi, Yoshihiro; Takasuga,
 Yoshihiro; Takaoka, Aya
CORPORATE SOURCE: National Institute of Health Sciences, 3-25-2
 Tonomachi, Kawasak-ku, Kawasaki, Kanagawa, 210-9501,
 Japan
SOURCE: Iyakuhin Iryo Kiki Regyuratori Saiensu (2018), 49(10),
 708-718

CODEN: IIKRA9; ISSN: 1884-6076
PUBLISHER: Nippon Koteisho Kyokai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB This study examines bacterial endotoxins using recombinant factor C-based procedure for detection of lipopolysaccharides. The reproducibility was evaluated by comparing the recombinant reagent and the lysate reagent. The measured inspection line was prepd. and the inspection line was prepd. using multiple prodn. lots of the recombinant reagent, and the recombinant reagent was evaluated from the obtained results.
OS.CITING REF COUNT: 2 THERE ARE 2 CAPLUS RECORDS THAT CITE THIS RECORD (2 CITINGS)
L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2020:817406 CAPLUS Full-text
DOCUMENT NUMBER: 174:115196
TITLE: Application of a recombinant three-factor chromogenic reagent, pyrosmart, for bacterial endotoxins test filed in the pharmacopeias
AUTHOR(S): Muroi, Masashi; Ogura, Norihiko; Mizumura, Hikaru; Aketagawa, Jun; Oda, Toshio; Tanamoto, Ken-ichi
CORPORATE SOURCE: Research Institute of Pharmaceutical Sciences, Musashino University, 1-1-20 Shinmachi, Nishitokyo, Tokyo, 202-8585, Japan
SOURCE: Biological & Pharmaceutical Bulletin (2019), 42(12), 2024-2037
CODEN: BPBLEO; ISSN: 0918-6158
DIGITAL OBJECT ID: 10.1248/bpb.b19-00517
PUBLISHER: Pharmaceutical Society of Japan
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Assays using lysate reagents prepd. from horseshoe crab hemocyte ext. (limulus amoebocyte lysate, LAL) are commonly and widely used to detect and measure endotoxin in parenteral drugs and medical devices. However, lysate reagents suffer from lot-to-lot variations leading to possible fluctuations in testing. Also, this continued usage of lysate reagents leads to the possible decline of the horseshoe crab population. Recently, a new recombinant chromogenic reagent, PyroSmart, consisting of three recombinant factors was introduced to the market. There are now three recombinant products; two with recombinant factor C reagents and PyroSmart with the complete recombinant LAL system. We evaluated the applicability of the reagent to the harmonized bacterial endotoxins test in the United States, European and Japanese pharmacopeias. The recombinant product showed equiv. potency of thirteen endotoxins from different bacterial strains to conventional chromogenic lysate reagents as long as their assay modes are identical. All anal. characteristics or assay parameters of the reagent satisfied the acceptance criteria which are set for the use for the bacterial endotoxins test filed in the pharmacopeias. All of 109 parenteral drugs tested can be measured with PyroSmart within resp. max. allowable dilns. The lot-to-lot variation in recovery of endotoxin added in the parenteral drugs for PyroSmart was equal to or less than those of six limulus lysate reagents. In conclusion, the present study suggests that the recombinant reagent, PyroSmart, provide a good alternative to the LAL reagents with better lot-to-lot variation.
OS.CITING REF COUNT: 5 THERE ARE 5 CAPLUS RECORDS THAT CITE THIS RECORD (5 CITINGS)
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2020:662542 CAPLUS Full-text
DOCUMENT NUMBER: 172:400963
TITLE: Preparation of recombinant proteins and reagents for detection of endotoxin from Helicobacter pylori with enhancing sensitivity
INVENTOR(S): Ogura, Norihiko; Kobayashi, Yuki; Mizumura, Hikaru; Oda, Toshio
PATENT ASSIGNEE(S): Seikagaku Corporation, Japan
ULTIMATE OWNER: SEIKAGAKU CORPORATION
ULTIMATE OWNER STANDARD: Seikagaku

SOURCE: PCT Int. Appl., 53pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2020071229	A1	20200409	WO 2019-JP37828	20190926
JP 6785521	B2	20201118	JP 2020-508631	20190926
CN 112639118	A	20210409	CN 2019-80056205	20190926
EP 3862437	A1	20210811	EP 2019-869215	20190926
US 20210215695	A1	20210715	US 2021-17272805	20210302
PRIORITY APPLN. INFO.:			JP 2018-188587	A 20181003
			WO 2019-JP37828	W 20190926

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2020071229	A1	Dead	20230601
JP 6785521	B2	Alive	20201127
CN 112639118	A	Alive	20210415
EP 3862437	A1	Alive	20210812
US 20210215695	A1	Alive	20210722

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB The present invention addresses the problem of providing a method for, in a conventional endotoxin measuring agent in which a recombinant protein is used, enhancing the sensitivity to a Helicobacter pylori-derived endotoxin. Provided is a method for enhancing the sensitivity to a Helicobacter pylori-derived endotoxin of an endotoxin measuring agent, said endotoxin measuring agent contg. a factor C recombinant protein derived from Tachypleus tridentatus, wherein the content of the factor C recombinant protein is increased to a level sufficient to enhance the sensitivity upon the measurement of the endotoxin.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
CN 112639118 A	CN 2019-80056205	Register Global Dossier
EP 3862437 A1	EP 2019-869215	Register Federated Reg.
JP 6785521 B2	JP 2020-508631	Register Global Dossier
US 20210215695 A1	US 2021-17272805	Register Global Dossier
WO 2020071229 A1	WO 2019-JP37828	Register Global Dossier

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2017:952137 CAPLUS Full-text

DOCUMENT NUMBER: 174:501931

TITLE: Genetic engineering approach to develop next-generation reagents for endotoxin quantification
AUTHOR(S): Mizumura, Hikaru; Ogura, Norihiko; Aketagawa, Jun; Aizawa, Maki; Kobayashi, Yuki; Kawabata, Shun-ichiro; Oda, Toshio

CORPORATE SOURCE: LAL Research and Development Department, Seikagaku Corporation, Tokyo, Japan

SOURCE: Innate Immunity (2017), 23(2), No pp. given

CODEN: IINMCB; ISSN: 1753-4259

DIGITAL OBJECT ID: 10.1177/1753425916681074

PUBLISHER: Sage Publications Ltd.

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English

AB The bacterial endotoxin test, which uses amebocyte lysate reagents of horseshoe crab origin, is a sensitive, reproducible and simple assay to measure endotoxin concn. To develop sustainable raw materials for lysate reagents that do not require horseshoe crabs, three recombinant protease zymogens (factor C, derived from mammalian cells; factor B; and the proclotting enzyme derived from insect cells) were prepd. using a genetic engineering technique. Recombinant cascade reagents (RCRs) were then prepd. to reconstruct the reaction cascade in the amebocyte lysate reagent. The protease activity of the RCR contg. recombinant factor C was much greater than that of recombinant factor C alone, indicating the efficiency of signal amplification in the cascade. Compared with the

RCR contg. the insect cell-derived factor C, those contg. mammalian cell-derived factor C, which features different glycosylation patterns, were less susceptible to interference by the injectable drug components. The std. curve of the RCR contg. mammalian cell-derived recombinant factor C had a steeper slope than the curves for those contg. natural lysate reagents, suggesting a greater sensitivity to endotoxin. The present study supports the future prodn. of recombinant reagents that do not require the use of natural resources.

OS.CITING REF COUNT: 5 THERE ARE 5 CAPLUS RECORDS THAT CITE THIS RECORD (5 CITINGS)
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2017:604917 CAPLUS Full-text
DOCUMENT NUMBER: 166:382593
TITLE: Transglutaminase-catalyzed incorporation of polyamines masks the DNA-binding region of the transcription factor Relish
AUTHOR(S): Maki, Kouki; Shibata, Toshio; Kawabata, Shun-ichiro
CORPORATE SOURCE: Graduate School of Systems Life Sciences, Faculty of Science, Kyushu University, Fukuoka, 819-0395, Japan
SOURCE: Journal of Biological Chemistry (2017), 292(15), 6369-6380
CODEN: JBCHA3; ISSN: 0021-9258
DIGITAL OBJECT ID: 10.1074/jbc.M117.779579
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English

AB In Drosophila, the final immune deficiency (IMD) pathway-dependent signal is transmitted through proteolytic conversion of the nuclear factor- κ B (NF- κ B)-like transcription factor Relish to the active N-terminal fragment Relish-N. Relish-N is then translocated from the cytosol into the nucleus for the expression of IMD-controlled genes. We previously demonstrated that transglutaminase (TG) suppresses the IMD pathway by polymg. Relish-N to inhibit its nuclear translocation. Conversely, we also demonstrated that orally ingested synthetic amines, such as monodansylcadaverine (DCA) and biotin-labeled pentylamine, are TG-dependently incorporated into Relish-N, causing the nuclear translocation of modified Relish-N in gut epithelial cells. It remains unclear, however, whether polyamine-contg. Relish-N retains transcriptional activity. Here, we used mass spectrometry anal. of a recombinant Relish-N modified with DCA by TG activity after proteolytic digestion and show that the DCA-modified Gln residues are located in the DNA-binding region of Relish-N. TG-catalyzed DCA incorporation inhibited binding of Relish-N to the Rel-responsive element in the NF- κ B-binding DNA sequence. Subcellular fractionation of TG-expressing Drosophila S2 cells indicated that TG was localized in both the cytosol and nucleus. Of note, natural polyamines, including spermidine and spermine, competitively inhibited TG-dependent DCA incorporation into Relish-N. Moreover, in vivo expts. demonstrated that Relish-N was modified by spermine and that this modification reduced transcription of IMD pathway-controlled cecropin A1 and dipterecin genes. These findings suggest that intracellular TG regulates Relish-N-mediated transcriptional activity by incorporating polyamines into Relish-N and via protein-protein crosslinking.

OS.CITING REF COUNT: 11 THERE ARE 11 CAPLUS RECORDS THAT CITE THIS RECORD (11 CITINGS)
REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2014:999086 CAPLUS Full-text
DOCUMENT NUMBER: 161:65883
TITLE: Novel recombinant factor C and method for producing same, and method for measuring endotoxin
INVENTOR(S): Mizumura, Hikaru; Oda, Toshio; Kawabata, Shun-ichiro
PATENT ASSIGNEE(S): Seikagaku Corporation, Japan
ULTIMATE OWNER: SEIKAGAKU CORPORATION
ULTIMATE OWNER STANDARD: Seikagaku
SOURCE: PCT Int. Appl., 79pp.

DOCUMENT TYPE:
 LANGUAGE:
 FAMILY ACC. NUM. COUNT:
 PATENT INFORMATION:

CODEN: PIXXD2
 Patent
 Japanese
 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2014092079	A1	20140619	WO 2013-JP83082	20131210
CN 104919046	A	20150916	CN 2013-80064649	20131210
EP 2930241	A1	20151014	EP 2013-862466	20131210
EP 2930241	B1	20190626		
SG 10201704774	A1	20170728	SG 2017-10201704774	20131210
CN 108646019	A	20181012	CN 2018-10481773	20131210
CN 108646019	B	20210625		
CN 108642030	A	20181012	CN 2018-10483329	20131210
EP 3441466	A1	20190213	EP 2018-190686	20131210
EP 3441466	B1	20200205		
JP 6511685	B2	20190515	JP 2014-552048	20131210
ES 2738593	T3	20200123	ES 2013-862466	20131210
EP 3663400	A1	20200610	EP 2019-216609	20131210
ES 2784510	T3	20200928	ES 2018-190686	20131210
EP 4219705	A1	20230802	EP 2022-215393	20131210
US 20150307864	A1	20151029	US 2015-14650767	20150609
US 10144923	B2	20181204		
US 20180258414	A1	20180913	US 2018-15983725	20180518
US 10982202	B2	20210420		
JP 2019000122	A	20190110	JP 2018-183630	20180928
JP 6626174	B2	20191225		
US 20190256835	A1	20190822	US 2019-16399575	20190430
US 20190270977	A1	20190905	US 2019-16399386	20190430
US 11236318	B2	20220201		
US 20220145280	A1	20220512	US 2021-17554128	20211217
PRIORITY APPLN. INFO.:			JP 2012-269840	A 20121210
			CN 2013-80064649	A3 20131210
			EP 2013-862466	A3 20131210
			EP 2018-190686	A3 20131210
			EP 2019-216609	A3 20131210
			JP 2014-552048	A3 20131210
			WO 2013-JP83082	W 20131210
			US 2015-14650767	A1 20150609
			US 2018-15983725	A1 20180518
			US 2019-16399386	A1 20190430

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2014092079	A1	Dead	20201201
CN 104919046	A	Dead	20201120
EP 2930241	A1	Indeterminate	20201107
EP 2930241	B1	Indeterminate	20201106
SG 10201704774	A1	Alive	20201120
CN 108646019	A	Alive	20201121
CN 108646019	B	Alive	20210708
CN 108642030	A	Alive	20201120
EP 3441466	A1	Indeterminate	20201106
EP 3441466	B1	Indeterminate	20201106
JP 6511685	B2	Alive	20201121
ES 2738593	T3	Alive	20201121
EP 3663400	A1	Alive	20201202
ES 2784510	T3	Alive	20201121
EP 4219705	A1	Alive	20230803
US 20150307864	A1	Alive	20201121
US 10144923	B2	Alive	20201120
US 20180258414	A1	Alive	20201121
US 10982202	B2	Alive	20210430
JP 2019000122	A	Alive	20201121
JP 6626174	B2	Alive	20201121
US 20190256835	A1	Dead	20210805
US 20190270977	A1	Alive	20201120
US 11236318	B2	Alive	20220210
US 20220145280	A1	Alive	20220519

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB Provided is a method for producing horseshoe crab recombinant factor

C. The horseshoe crab recombinant factor C can be produced by expressing the horseshoe crab factor C using a mammalian cell such as CHO DG44 and HEK293 as a host cell.

OS.CITING REF COUNT: 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
CN 104919046 A	CN 2013-80064649	Register Global Dossier
CN 108646019 A	CN 2018-10481773	Register Global Dossier
CN 108646019 B	CN 2018-10481773	Register Global Dossier
CN 108642030 A	CN 2018-10483329	Register Global Dossier
EP 2930241 A1	EP 2013-862466	Register Federated Reg.
EP 2930241 B1	EP 2013-862466	Register Federated Reg.
EP 3441466 A1	EP 2018-190686	Register Federated Reg.
EP 3441466 B1	EP 2018-190686	Register Federated Reg.
EP 3663400 A1	EP 2019-216609	Register Federated Reg.
EP 4219705 A1	EP 2022-215393	Register Federated Reg.
ES 2738593 T3	ES 2013-862466	Register
ES 2784510 T3	ES 2018-190686	Register
JP 6511685 B2	JP 2014-552048	Register Global Dossier
JP 2019000122 A	JP 2018-183630	Register Global Dossier
JP 6626174 B2	JP 2018-183630	Register Global Dossier
SG 10201704774 A1	SG 2017-10201704774	Register
US 10144923 B2	US 2015-14650767	Register Global Dossier
US 20150307864 A1	US 2015-14650767	Register Global Dossier
US 10982202 B2	US 2018-15983725	Register Global Dossier
US 20180258414 A1	US 2018-15983725	Register Global Dossier
US 11236318 B2	US 2019-16399386	Register Global Dossier
US 20190270977 A1	US 2019-16399386	Register Global Dossier
US 20190256835 A1	US 2019-16399575	Register Global Dossier
US 20220145280 A1	US 2021-17554128	Register Global Dossier
WO 2014092079 A1	WO 2013-JP83082	Register Global Dossier

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2012:1302054 CAPLUS Full-text

DOCUMENT NUMBER: 157:457279

TITLE: An agent for measuring endotoxin comprised of recombinant proteins expressed in insect cells

INVENTOR(S): Mizumura, Hikaru; Aizawa, Maki; Oda, Toshio

PATENT ASSIGNEE(S): Seikagaku Corporation, Japan

ULTIMATE OWNER: SEIKAGAKU CORPORATION

ULTIMATE OWNER STANDARD: Seikagaku

SOURCE: PCT Int. Appl., 72pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2012118226	A1	20120907	WO 2012-JP55728	20120228
EP 2681564	A1	20140108	EP 2012-710808	20120228
EP 2681564	B1	20171025		
CN 103562724	A	20140205	CN 2012-80010708	20120228
CN 103562724	B	20161005		
JP 2014510898	T	20140501	JP 2013-538748	20120228
SG 10201601351	A1	20160330	SG 2016-10201601351	20120228
CN 106432457	A	20170222	CN 2016-10799888	20120228
ES 2650170	T3	20180117	ES 2012-710808	20120228
EP 3273244	A1	20180124	EP 2017-190094	20120228
EP 3273244	B1	20190925		
EP 3517962	A1	20190731	EP 2019-160846	20120228
ES 2763089	T3	20200527	ES 2017-190094	20120228
SG 10202110031	A1	20211028	SG 2021-10202110031	20120228
US 20140024062	A1	20140123	US 2013-14001138	20130822
US 20150252405	A1	20150910	US 2015-14704230	20150505
US 20170138945	A1	20170518	US 2016-15359058	20161122
JP 2018141797	A	20180913	JP 2018-74569	20180409

JP 6640267	B2	20200205		
US 20180259527	A1	20180913	US 2018-15984288	20180518
US 11162949	B2	20211102		
JP 2020100622	A	20200702	JP 2019-233842	20191225
US 20220018841	A1	20220120	US 2021-17449736	20211001
JP 2022119887	A	20220817	JP 2022-85191	20220525
PRIORITY APPLN. INFO.:			US 2011-61447556	P 20110228
			CN 2012-80010708	A3 20120228
			EP 2012-710808	A3 20120228
			EP 2017-190094	A3 20120228
			JP 2013-538748	A3 20120228
			WO 2012-JP55728	W 20120228
			US 2013-14001138	A1 20130822
			US 2015-14704230	B1 20150505
			US 2016-15359058	A1 20161122
			JP 2018-74569	A3 20180409
			US 2018-15984288	A1 20180518
			JP 2019-233842	A3 20191225

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2012118226	A1	Dead	20201202
EP 2681564	A1	Indeterminate	20201106
EP 2681564	B1	Indeterminate	20201106
CN 103562724	A	Alive	20201120
CN 103562724	B	Alive	20201121
JP 2014510898	T	Alive	20201120
SG 10201601351	A1	Alive	20201121
CN 106432457	A	Dead	20210318
ES 2650170	T3	Alive	20201120
EP 3273244	A1	Indeterminate	20201106
EP 3273244	B1	Indeterminate	20201107
EP 3517962	A1	Alive	20201201
ES 2763089	T3	Alive	20201121
SG 10202110031	A1	Alive	20211125
US 20140024062	A1	Dead	20201121
US 20150252405	A1	Dead	20201121
US 20170138945	A1	Dead	20201121
JP 2018141797	A	Alive	20201120
JP 6640267	B2	Alive	20201121
US 20180259527	A1	Alive	20210506
US 11162949	B2	Alive	20211124
JP 2020100622	A	Alive	20201120
US 20220018841	A1	Alive	20220127
JP 2022119887	A	Alive	20220901

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB A method for rapidly and highly sensitively measuring endotoxin is provided. Endotoxin is measured using an endotoxin-measuring agent comprising the proteins (1) to (3) below, each of which is a recombinant protein obtainable by being expressed using insect cells as a host: (1) a factor C derived from Tachyplesus tridentatus, which factor C does not have a His-tag sequence at the C-terminus; (2) a factor B of a horseshoe crab; and (3) a proclotting enzyme of a horseshoe crab.

OS.CITING REF COUNT: 6 THERE ARE 6 CAPLUS RECORDS THAT CITE THIS RECORD (6 CITINGS)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
CN 103562724 A	CN 2012-80010708	Register Global Dossier
CN 103562724 B	CN 2012-80010708	Register Global Dossier
CN 106432457 A	CN 2016-10799888	Register Global Dossier
EP 2681564 A1	EP 2012-710808	Register Federated Reg.
EP 2681564 B1	EP 2012-710808	Register Federated Reg.
EP 3273244 A1	EP 2017-190094	Register Federated Reg.
EP 3273244 B1	EP 2017-190094	Register Federated Reg.
EP 3517962 A1	EP 2019-160846	Register Federated Reg.
ES 2650170 T3	ES 2012-710808	Register
ES 2763089 T3	ES 2017-190094	Register
JP 2014510898 T	JP 2013-538748	Register Global Dossier

JP 2018141797 A	JP 2018-74569	Register Global Dossier
JP 6640267 B2	JP 2018-74569	Register Global Dossier
JP 2020100622 A	JP 2019-233842	Register Global Dossier
JP 2022119887 A	JP 2022-85191	Register Global Dossier
SG 10201601351 A1	SG 2016-10201601351	Register
SG 10202110031 A1	SG 2021-10202110031	Register
US 20140024062 A1	US 2013-14001138	Register Global Dossier
US 20150252405 A1	US 2015-14704230	Register Global Dossier
US 20170138945 A1	US 2016-15359058	Register Global Dossier
US 11162949 B2	US 2018-15984288	Register Global Dossier
US 20180259527 A1	US 2018-15984288	Register Global Dossier
US 20220018841 A1	US 2021-17449736	Register Global Dossier
WO 2012118226 A1	WO 2012-JP55728	Register Global Dossier

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 1994:624482 CAPLUS Full-text

DOCUMENT NUMBER: 121:224482

ORIGINAL REFERENCE NO.: 121:40761a,40764a

TITLE: cDNA cloning and characterization of mitochondrial import stimulation factor (MSF) purified from rat liver cytosol

AUTHOR(S): Alam, Rashidul; Hachiya, Naomi; Sakaguchi, Masao; Kawabata, Shun-ichiro; Iwanaga, Sadaaki; Kitajima, Masato; Mihara, Katsuyoshi; Omura, Tsuneo

CORPORATE SOURCE: Graduate School Medical Science, Kyushu University, Fukuoka, 812, Japan

SOURCE: Journal of Biochemistry (1994), 116(2), 416-25

CODEN: JOBIAO; ISSN: 0021-924X

DIGITAL OBJECT ID: 10.1093/oxfordjournals.jbchem.a124541

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors identified a liver cytosolic protein factor that stimulated the import of wheat germ lysate-synthesized precursor proteins into mitochondria. It was termed mitochondrial import stimulation factor or MSF [Hachiya, N. et al. (1993) EMBO J. 12, 1579-1586]. It consisted of 32-kDa (MSFL) and 30-kDa (MSFS) polypeptides as assessed by SDS-PAGE. MSF recognized the presequence portion of mitochondrial precursor proteins and catalyzed the depolymn. and unfolding of in vitro synthesized mitochondrial precursor proteins in an ATP-dependent manner. The authors report here the cDNA cloning and characterization of MSF. Microsequencing of MSFL and MSFS showed that they belonged to a highly conserved, widely distributed eukaryotic protein family, collectively designated as 14-3-3 proteins. The authors cloned the cDNA of MSFL and that of one component of MSFS (MSFS1) from a rat liver cDNA library. The cloned cDNAs were sep. expressed in Escherichia coli and the expressed proteins were purified to homogeneity. The purified recombinant MSFL and MSFS1 stimulated mitochondrial import of adrenodoxin precursor (pAd) synthesized in vitro with wheat germ lysate translation system. Recombinant MSFL and MSFS1 had the ability to bind with denatured pAd and they kept the precursor in an import-competent state. Rabbit polyclonal antibodies raised against the recombinant proteins inhibited the import-stimulation activity of rat liver cytosol as well as that of authentic purified MSF. Identification of MSF as 14-3-3 proteins establishes a novel function for this family of proteins and indicates their role as cytosolic chaperones to aid many important cellular events.

OS.CITING REF COUNT: 66 THERE ARE 66 CAPLUS RECORDS THAT CITE THIS RECORD (66 CITINGS)

=> d his

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FILE 'CAPLUS' ENTERED AT 05:42:36 ON 12 SEP 2023

	E MIZUMURA HIKARU/AU 25
L1	26 S E3
	E ODA TOSHIO/AU 25
L2	94 S E3
	E KAWABATA SHUN/AU 25
L3	144 S E4

```

L4          247 S L1 OR L2 OR L3
L5      3193236 S FACTOR
L6          72 S L4 AND L5
L7      949971 S (SECRET? OR EXCRET?)
L8          8 S L6 AND L7
L9          9 S L6 AND RECOMBINANT
L10         9 S L9 NOT L8

```

=> s l6 and (CHO OR HEK?)

```

      82151 CHO
      59175 HEK?
L11      3 L6 AND (CHO OR HEK?)

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=> s l6 and (chinese OR kidney)

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      608400 CHINESE
      707510 KIDNEY
L12      0 L6 AND (CHINESE OR KIDNEY)

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=> d ibib abs 1- l11

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

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L11 ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER:    2015:1262044  CAPLUS Full-text
DOCUMENT NUMBER:     163:452297
TITLE:               Factor B Is the Second Lipopolysaccharide-binding
                        Protease Zymogen in the Horseshoe Crab Coagulation
                        Cascade
AUTHOR(S):           Kobayashi, Yuki; Takahashi, Toshiaki; Shibata, Toshio;
                        Ikeda, Shunsuke; Koshiba, Takumi; Mizumura, Hikaru;
                        Oda, Toshio; Kawabata, Shun-ichiro
CORPORATE SOURCE:    Department of Biology, Faculty of Sciences, Kyushu
                        University, Fukuoka, 812-8581, Japan
SOURCE:              Journal of Biological Chemistry (2015), 290(31),
                        19379-19386
                        CODEN: JBCHA3; ISSN: 0021-9258
DIGITAL OBJECT ID:   10.1074/jbc.M115.653196
PUBLISHER:           American Society for Biochemistry and Molecular
                        Biology
DOCUMENT TYPE:        Journal; (online computer file)
LANGUAGE:             English

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AB  Factor B is a serine-protease zymogen in the horseshoe crab coagulation
    cascade, and it is the primary substrate for activated factor C, the
    LPS-responsive initiator of the cascade. Factor C is autocatalytically
    activated to  $\alpha$ -factor C on LPS and is artificially converted to
     $\beta$ -factor C, another activated form, by chymotrypsin. It is not
    known, however, whether LPS is required for the activation of factor B.
    Here we found that wild-type factor B expressed in HEK293S cells is
    activated by  $\alpha$ -factor C, but not by  $\beta$ -factor C, in an
    LPS-dependent manner and that  $\beta$ -factor C loses the LPS binding
    activity of factor C through addnl. cleavage by chymotrypsin within the
    N-terminal LPS-binding region. Surface plasmon resonance and quartz
    crystal microbalance analyses revealed that wild-type factor B binds to
    LPS with high affinity comparable to that of factor C, demonstrating
    that factor B is the second LPS-binding zymogen in the cascade. An
    LPS-binding site of wild-type factor B was found in the N-terminal clip
    domain, and the activation rate of a clip domain deletion mutant was
    considerably slower than that of wild-type factor B. Moreover, in the
    presence of LPS, Triton X-100 inhibited the activation of wild-type
    factor B by  $\alpha$ -factor C. We conclude that the clip domain of
    factor B has an important role in localizing factor B to the surface
    of Gram-neg. bacteria or LPS released from bacteria to initiate effective
    proteolytic activation by  $\alpha$ -factor C.
OS.CITING REF COUNT:  9      THERE ARE 9 CAPLUS RECORDS THAT CITE THIS RECORD
                        (9 CITINGS)
REFERENCE COUNT:      29     THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS
                        RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

```

L11 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2014:1539054 CAPLUS Full-text
 DOCUMENT NUMBER: 161:468700
 TITLE: The N-terminal Arg Residue Is Essential for Autocatalytic Activation of a Lipopolysaccharide-responsive Protease Zymogen
 AUTHOR(S): Kobayashi, Yuki; Shiga, Takafumi; Shibata, Toshio; Sako, Miyuki; Maenaka, Katsumi; Koshiba, Takumi; Mizumura, Hikaru; Oda, Toshio; Kawabata, Shun-ichiro
 CORPORATE SOURCE: Graduate School of Systems Life Sciences, Kyushu University, 812-8581, Japan
 SOURCE: Journal of Biological Chemistry (2014), 289(37), 25987-25995
 CODEN: JBCHA3; ISSN: 0021-9258
 DIGITAL OBJECT ID: 10.1074/jbc.M114.586933
 PUBLISHER: American Society for Biochemistry and Molecular Biology
 DOCUMENT TYPE: Journal; (online computer file)
 LANGUAGE: English
 AB Factor C, a serine protease zymogen involved in innate immune responses in horseshoe crabs, is known to be autocatalytically activated on the surface of bacterial lipopolysaccharides, but the mol. mechanism of this activation remains unknown. In this study, we show that wild-type factor C expressed in HEK293S cells exhibits a lipopolysaccharide-induced activity equiv. to that of native factor C. Anal. of the N-terminal addn., deletion, or substitution mutants shows that the N-terminal Arg residue and the distance between the N terminus and the tripartite lipopolysaccharide-binding site are essential factors for autocatalytic activation, and that the pos. charge of the N terminus may interact with an acidic amino acid(s) of the mol. to convert the zymogen into an active form. Chem. crosslinking expts. indicate that the N terminus is required to form a complex of the factor C mols. in a sufficiently close vicinity to be chem. cross-linked on the surface of lipopolysaccharides. We propose a mol. mechanism of the autocatalytic activation of the protease zymogen on lipopolysaccharides functioning as a platform to induce specific protein-protein interaction between the factor C mols.
 OS.CITING REF COUNT: 11 THERE ARE 11 CAPLUS RECORDS THAT CITE THIS RECORD (11 CITINGS)
 REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2014:999086 CAPLUS Full-text
 DOCUMENT NUMBER: 161:65883
 TITLE: Novel recombinant factor C and method for producing same, and method for measuring endotoxin
 INVENTOR(S): Mizumura, Hikaru; Oda, Toshio; Kawabata, Shun-ichiro
 PATENT ASSIGNEE(S): Seikagaku Corporation, Japan
 ULTIMATE OWNER: SEIKAGAKU CORPORATION
 ULTIMATE OWNER STANDARD: Seikagaku
 SOURCE: PCT Int. Appl., 79pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2014092079	A1	20140619	WO 2013-JP83082	20131210
CN 104919046	A	20150916	CN 2013-80064649	20131210
EP 2930241	A1	20151014	EP 2013-862466	20131210
EP 2930241	B1	20190626		
SG 10201704774	A1	20170728	SG 2017-10201704774	20131210
CN 108646019	A	20181012	CN 2018-10481773	20131210
CN 108646019	B	20210625		
CN 108642030	A	20181012	CN 2018-10483329	20131210
EP 3441466	A1	20190213	EP 2018-190686	20131210
EP 3441466	B1	20200205		
JP 6511685	B2	20190515	JP 2014-552048	20131210
ES 2738593	T3	20200123	ES 2013-862466	20131210
EP 3663400	A1	20200610	EP 2019-216609	20131210
ES 2784510	T3	20200928	ES 2018-190686	20131210

EP 4219705	A1	20230802	EP 2022-215393	20131210
US 20150307864	A1	20151029	US 2015-14650767	20150609
US 10144923	B2	20181204		
US 20180258414	A1	20180913	US 2018-15983725	20180518
US 10982202	B2	20210420		
JP 2019000122	A	20190110	JP 2018-183630	20180928
JP 6626174	B2	20191225		
US 20190256835	A1	20190822	US 2019-16399575	20190430
US 20190270977	A1	20190905	US 2019-16399386	20190430
US 11236318	B2	20220201		
US 20220145280	A1	20220512	US 2021-17554128	20211217
PRIORITY APPLN. INFO.:			JP 2012-269840	A 20121210
			CN 2013-80064649	A3 20131210
			EP 2013-862466	A3 20131210
			EP 2018-190686	A3 20131210
			EP 2019-216609	A3 20131210
			JP 2014-552048	A3 20131210
			WO 2013-JP83082	W 20131210
			US 2015-14650767	A1 20150609
			US 2018-15983725	A1 20180518
			US 2019-16399386	A1 20190430

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2014092079	A1	Dead	20201201
CN 104919046	A	Dead	20201120
EP 2930241	A1	Indeterminate	20201107
EP 2930241	B1	Indeterminate	20201106
SG 10201704774	A1	Alive	20201120
CN 108646019	A	Alive	20201121
CN 108646019	B	Alive	20210708
CN 108642030	A	Alive	20201120
EP 3441466	A1	Indeterminate	20201106
EP 3441466	B1	Indeterminate	20201106
JP 6511685	B2	Alive	20201121
ES 2738593	T3	Alive	20201121
EP 3663400	A1	Alive	20201202
ES 2784510	T3	Alive	20201121
EP 4219705	A1	Alive	20230803
US 20150307864	A1	Alive	20201121
US 10144923	B2	Alive	20201120
US 20180258414	A1	Alive	20201121
US 10982202	B2	Alive	20210430
JP 2019000122	A	Alive	20201121
JP 6626174	B2	Alive	20201121
US 20190256835	A1	Dead	20210805
US 20190270977	A1	Alive	20201120
US 11236318	B2	Alive	20220210
US 20220145280	A1	Alive	20220519

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB Provided is a method for producing horseshoe crab recombinant factor C. The horseshoe crab recombinant factor C can be produced by expressing the horseshoe crab factor C using a mammalian cell such as CHO DG44 and HEK293 as a host cell.

OS.CITING REF COUNT: 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
CN 104919046 A	CN 2013-80064649	Register Global Dossier
CN 108646019 A	CN 2018-10481773	Register Global Dossier
CN 108646019 B	CN 2018-10481773	Register Global Dossier
CN 108642030 A	CN 2018-10483329	Register Global Dossier
EP 2930241 A1	EP 2013-862466	Register Federated Reg.
EP 2930241 B1	EP 2013-862466	Register Federated Reg.
EP 3441466 A1	EP 2018-190686	Register Federated Reg.
EP 3441466 B1	EP 2018-190686	Register Federated Reg.
EP 3663400 A1	EP 2019-216609	Register Federated Reg.
EP 4219705 A1	EP 2022-215393	Register Federated Reg.

ES 2738593 T3	ES 2013-862466	Register
ES 2784510 T3	ES 2018-190686	Register
JP 6511685 B2	JP 2014-552048	Register Global Dossier
JP 2019000122 A	JP 2018-183630	Register Global Dossier
JP 6626174 B2	JP 2018-183630	Register Global Dossier
SG 10201704774 A1	SG 2017-10201704774	Register
US 10144923 B2	US 2015-14650767	Register Global Dossier
US 20150307864 A1	US 2015-14650767	Register Global Dossier
US 10982202 B2	US 2018-15983725	Register Global Dossier
US 20180258414 A1	US 2018-15983725	Register Global Dossier
US 11236318 B2	US 2019-16399386	Register Global Dossier
US 20190270977 A1	US 2019-16399386	Register Global Dossier
US 20190256835 A1	US 2019-16399575	Register Global Dossier
US 20220145280 A1	US 2021-17554128	Register Global Dossier
WO 2014092079 A1	WO 2013-JP83082	Register Global Dossier

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COST IN U.S. DOLLARS

SINCE FILE
ENTRY
333.15

TOTAL
SESSION
333.43

FILE 'MEDLINE' ENTERED AT 05:51:39 ON 12 SEP 2023

FILE 'AGRICOLA' ENTERED AT 05:51:39 ON 12 SEP 2023

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FILE 'CAPLUS' ENTERED AT 05:51:39 ON 12 SEP 2023

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FILE 'SCISEARCH' ENTERED AT 05:51:39 ON 12 SEP 2023

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=> s "factor c"

L13 95203 "FACTOR C"

=> s (CHO OR HEK?)

L14 671016 (CHO OR HEK?)

=> s (chinese OR kidney)

L15 6689580 (CHINESE OR KIDNEY)

=> s l14 or l15

L16 7108972 L14 OR L15

=> s l13 and l16

L17 5621 L13 AND L16

=> s l17 and recombinant?

L18 258 L17 AND RECOMBINANT?

=> s (secret? OR excret?)

L19 5389518 (SECRET? OR EXCRET?)

=> s l18 and l19

L20 37 L18 AND L19

=> dup rem l20

PROCESSING COMPLETED FOR L20

L21 29 DUP REM L20 (8 DUPLICATES REMOVED)

=> d his

(FILE 'HOME' ENTERED AT 05:42:13 ON 12 SEP 2023)

FILE 'CAPLUS' ENTERED AT 05:42:36 ON 12 SEP 2023

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L1 26 S E3
E ODA TOSHIO/AU 25
L2 94 S E3
E KAWABATA SHUN/AU 25
L3 144 S E4
L4 247 S L1 OR L2 OR L3
L5 3193236 S FACTOR
L6 72 S L4 AND L5
L7 949971 S (SECRET? OR EXCRET?)
L8 8 S L6 AND L7
L9 9 S L6 AND RECOMBINANT
L10 9 S L9 NOT L8
L11 3 S L6 AND (CHO OR HEK?)
L12 0 S L6 AND (CHINESE OR KIDNEY)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT
05:51:39 ON 12 SEP 2023

L13 95203 S "FACTOR C"
L14 671016 S (CHO OR HEK?)
L15 6689580 S (CHINESE OR KIDNEY)
L16 7108972 S L14 OR L15
L17 5621 S L13 AND L16
L18 258 S L17 AND RECOMBINANT?
L19 5389518 S (SECRET? OR EXCRET?)
L20 37 S L18 AND L19
L21 29 DUP REM L20 (8 DUPLICATES REMOVED)

=> s l21 not (l8 or l9)

L22 29 L21 NOT (L8 OR L9)

=> d ibib abs 1-

YOU HAVE REQUESTED DATA FROM 29 ANSWERS - CONTINUE? Y/(N):y

L22 ANSWER 1 OF 29 MEDLINE @ on STN
ACCESSION NUMBER: 2021123288 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 31054183
TITLE: Amelioration of Endoplasmic Reticulum Stress by Mesenchymal
Stem Cells via Hepatocyte Growth Factor/c-Met Signaling
in Obesity-Associated Kidney Injury.
AUTHOR: Li Bin; Leung Joseph C K; Chan Loretta Y Y; Yiu Wai Han; Li
Ye; Lok Sarah W Y; Liu Wing Han; Chan Kam Wa; Lai Kar Neng;
Tang Sydney C W
AUTHOR IDENTIFIERS: ORCID: <https://orcid.org/0000-0002-6862-1941>
CORPORATE SOURCE: Division of Nephrology, Department of Medicine, The
University of Hong Kong, Queen Mary Hospital, Hong Kong,
People's Republic of China.
AUTHOR: Tse Hung Fat
CORPORATE SOURCE: Division of Cardiology, Department of Medicine, The
University of Hong Kong, Queen Mary Hospital, Hong Kong,
People's Republic of China.
SOURCE: Stem cells translational medicine, (20190900) Vol. 8, No.
9, pp. 898-910. Electronic Publication Date: 3 May 2019
Journal code: 101578022. E-ISSN: 2157-6580. L-ISSN:

2157-6564.
 Report No.: PMC-PMC6708066.
 DIGITAL OBJECT ID: <http://dx.doi.org/10.1002/sctm.18-0265>
 PUB. COUNTRY: England: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 (RESEARCH SUPPORT, NON-U.S. GOV'T)
 LANGUAGE: English
 FILE SEGMENT: MEDLINE; Priority Journals
 FILE SEGMENT: Print; Electronic
 ENTRY MONTH: 202006
 ENTRY DATE: Entered STN: 7 May 2019
 Last Updated on STN: 26 Jun 2020
 Indexing Added: 26 Jun 2020
 Entered Medline: 25 Jun 2020
 OS.CITING REF COUNT: 16 There are 16 MEDLINE records that cite this record
 MEDLINE REFERENCE COUNT: 55 There are 55 cited references available in
 MEDLINE for this document.

AB Recent advances in the understanding of lipid metabolism suggest a critical role of endoplasmic reticulum (ER) stress in obesity-induced kidney injury. Hepatocyte growth factor (HGF) is a pleiotropic cytokine frequently featured in stem cell therapy with distinct renotropic benefits. This study aims to define the potential link between human induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSCs)/bone marrow-derived MSCs (BM-MSCs) and ER stress in lipotoxic kidney injury induced by palmitic acid (PA) in renal tubular cells and by high-fat diet (HFD) in mice. iPS-MSCs or BM-MSCs alleviated ER stress (by preventing induction of Bip, chop, and unfolded protein response), inflammation (Il6, Cxcl1, and Cxcl2), and apoptosis (Bax/Bcl2 and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling-positive cells) in renal cortex of animals exposed to HFD thus mitigating histologic damage and albuminuria, via activating HGF/c-Met paracrine signaling that resulted in enhanced HGF secretion in the glomerular compartment and c-Met expression in the tubules. Coculture experiments identified glomerular endothelial cells (GECs) to be the exclusive source of glomerular HGF when incubated with either iPS-MSCs or BM-MSCs in the presence of PA. Furthermore, both GEC-derived HGF and exogenous recombinant HGF attenuated PA-induced ER stress in cultured tubular cells, and this effect was abrogated by a neutralizing anti-HGF antibody. Taken together, this study is the first to demonstrate that MSCs ameliorate lipotoxic kidney injury via a novel microenvironment-dependent paracrine HGF/c-Met signaling mechanism to suppress ER stress and its downstream pro-inflammatory and pro-apoptotic consequences. Stem Cells Translational Medicine 2019;8:898&910.
 © 2019 The Authors. Stem Cells Translational Medicine published by Wiley Periodicals, Inc. on behalf of AlphaMed Press.

L22 ANSWER 2 OF 29 MEDLINE @ on STN
 ACCESSION NUMBER: 2008510788 MEDLINE Full-text
 DOCUMENT NUMBER: PubMed ID: 18588873
 TITLE: Identification and expression analysis of an N-terminally truncated isoform of human PDGF-C.
 AUTHOR: Zhao Jian
 CORPORATE SOURCE: Department of Oncology-Pathology, Karolinska Institutet, CCK R8:05, Karolinska University Hospital, SE-171 76 Stockholm, Sweden. Jian.Zhao@ki.se
 AUTHOR: Liu Zhaoxu; Liu Tong; Nilsson Sten; Nister Monica
 SOURCE: Experimental cell research, (2008 Aug 15) Vol. 314, No. 14, pp. 2529-43. Electronic Publication Date: 13 Jun 2008
 Journal code: 0373226. E-ISSN: 1090-2422. L-ISSN: 0014-4827.
 DIGITAL OBJECT ID: <http://dx.doi.org/10.1016/j.yexcr.2008.06.007>
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 (RESEARCH SUPPORT, NON-U.S. GOV'T)
 LANGUAGE: English
 FILE SEGMENT: MEDLINE; Priority Journals
 FILE SEGMENT: Print; Electronic
 OTHER SOURCE: GENBANK-AM922296
 ENTRY MONTH: 200809
 ENTRY DATE: Entered STN: 13 Aug 2008
 Last Updated on STN: 16 Sep 2008
 Entered Medline: 15 Sep 2008

OS.CITING REF COUNT: 2 There are 2 MEDLINE records that cite this record

AB Platelet-derived growth factor C (PDGF-C) is a member of the PDGF family that plays an important role in developmental and physiological processes, and in human diseases. Here, we report a novel splice variant of human PDGF-C (PDGF-Cb), which encodes an N-terminally truncated protein, lacking the signal peptide and CUB domain. This variant is coexpressed with PDGF-C in all normal tissues analyzed. PDGF-Cb is produced as a cytoplasmic protein, and has a similar intracellular localization to PDGF-C, but is not secreted from transfected cells. Further, we show that PDGF-Cb can form heterodimers (PDGF-CCb) with PDGF-C, which is thereby retained and degraded within cells. In primary renal cell carcinoma (RCC), expression of the two alternatively spliced transcripts was different. Generally, expression of the full-length PDGF-C transcript was increased in RCC tumors, whereas expression of PDGF-Cb was not in the 30 analyzed cases with paired RCC tumor tissues and normal renal tissues. Based on these findings, we suggest that PDGF-Cb might act as a dominant negative molecule regulating the secretion of PDGF-C, and that deregulation of full-length PDGF-C is involved in RCC tumorigenesis.

L22 ANSWER 3 OF 29 MEDLINE @ on STN

ACCESSION NUMBER: 2005488650 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16159421

TITLE: Effect of recombinant VEGF-C secreted from eukaryotic cells on proliferation and chemotherapy-induced apoptosis of leukemic cells.

AUTHOR: Ding Kai-Yang

CORPORATE SOURCE: Jiangsu Institute of Hematology, The First Affiliated Hospital, Soochow University, Suzhou, Jiangsu, P.R. China.

AUTHOR: Bai Xia; Dai Lan; Dong Ning-Zheng; Ruan Chang-Geng

SOURCE: Ai zheng = Aizheng = Chinese journal of cancer, (2005 Sep) Vol. 24, No. 9, pp. 1037-42.

Journal code: 9424852.

PUB. COUNTRY: China

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: Chinese

FILE SEGMENT: MEDLINE; Priority Journals

FILE SEGMENT: Print

ENTRY MONTH: 200704

ENTRY DATE: Entered STN: 15 Sep 2005

Last Updated on STN: 15 Dec 2005

Entered Medline: 20 Apr 2007

AB BACKGROUND & OBJECTIVE: Vascular endothelial growth factor-C/vascular endothelial growth factor receptor-2, -3 (VEGF-C/VEGFR-2,3) signaling pathway can induce angiogenesis/lymphangiogenesis, and promote invasion and metastasis of solid tumors. Recent studies showed that VEGF-C and its receptors also expressed on hematological tumor cells, but their effects on leukemic cells are unclear. This study was to explore the effects of recombinant VEGF-C secreted from VEGF-C cDNA-transfected CHO (CHO/VEGF-C) cells on proliferation and chemotherapy-induced apoptosis of leukemic cells.

METHODS: The effect of recombinant VEGF-C (in the supernatants of CHO/VEGF-C cells) on angiogenesis was observed using the chicken chorioallantoic membrane model. The expression of VEGFR-2 and VEGFR-3 on leukemic cell lines TF-1, K562, HEL, NB4, HL-60, MR2, U937, SHI-1, and RPMI8226 was detected by reverse transcription-polymerase chain reaction (RT-PCR) and flow cytometry (FCM). The effect of VEGF-C on the proliferation of NB4 and K562 cells was assessed by MTT assay; its effect on the apoptosis of NB4 cells, which were treated with chemotherapeutic agents [etoposide (VP-16), daunorubicin (DNR), or arsenic trioxide (As2O3)], was investigated by FCM with Annexin V/PI double staining. The supernatant of CHO/pcDNA3.1 cells was used as control.

RESULTS: The recombinant VEGF-C induced angiogenesis in the chicken chorioallantoic membrane model. VEGFR-3 was expressed in NB4, HEL, and RPMI8226 cells; VEGFR-2 was expressed in HEL and TF-1 cells. Compared with the controls, the recombinant VEGF-C promoted the proliferation of NB4 cells (VEGFR-3(+)) (P(24h)=0.006, P(48h)=0.018), but had no effect on K562 cells (VEGFR-3(-)); it inhibited the chemotherapy-induced apoptosis of NB4 cells (P(VP-16)=0.019, P(DNR)=0.013, P(As2O3)=0.042).

CONCLUSION: The recombinant VEGF-C could induce angiogenesis, and may promote the proliferation and inhibit the chemotherapy-induced apoptosis of leukemic cells via VEGFR-3 signaling pathway, which might be potential target therapy for leukemia.

L22 ANSWER 4 OF 29 MEDLINE @ on STN
ACCESSION NUMBER: 2003257194 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 12782675
TITLE: The secretory proprotein convertases furin, PC5, and PC7 activate VEGF-C to induce tumorigenesis.
AUTHOR: Siegfried Geraldine
CORPORATE SOURCE: Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada.
AUTHOR: Basak Ajoy; Cromlish James A; Benjannet Suzanne; Marcinkiewicz Jadwiga; Chretien Michel; Seidah Nabil G; Khatib Abdel-Majid
SOURCE: The Journal of clinical investigation, (2003 Jun) Vol. 111, No. 11, pp. 1723-32.
Journal code: 7802877. ISSN: 0021-9738. L-ISSN: 0021-9738. Report No.: PMC-PMC156106.
DIGITAL OBJECT ID: <http://dx.doi.org/10.1172/JCI17220>
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: MEDLINE; Priority Journals
FILE SEGMENT: Print
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 4 Jun 2003
Last Updated on STN: 13 Jul 2003
Entered Medline: 11 Jul 2003

OS.CITING REF COUNT: 83 There are 83 MEDLINE records that cite this record
MEDLINE REFERENCE COUNT: 42 There are 42 cited references available in MEDLINE for this document.

AB The secretory factor VEGF-C has been directly implicated in various physiological processes during embryogenesis and human cancers. However, the importance of the conversion of its precursor proVEGF-C to mature VEGF-C in tumorigenesis, and vessel formation and the identity of the protease(s) that regulate these processes is/are not known. The intracellular processing of proVEGF-C that occurs within the dibasic motif HSIIRR(227)SL suggests the involvement of the proprotein convertases (PCs) in this process. In addition, furin and VEGF-C were found to be coordinately expressed in adult mouse tissues. Cotransfection of the furin-deficient colon carcinoma cell line LoVo with proVEGF-C and different PC members revealed that furin, PC5, and PC7 are candidate VEGF-C convertases. This finding is consistent with the in vitro digestions of an internally quenched synthetic fluorogenic peptide mimicking the cleavage site of proVEGF-C ((220)Q-VHSIIRR downward arrow SLP(230)). The processing of proVEGF-C is blocked by the inhibitory prosegments of furin, PC5, and PACE4, as well as by furin-motif variants of alpha2-macroglobulin and alpha1-antitrypsin. Subcutaneous injection of CHO cells stably expressing VEGF-C into nude mice enhanced angiogenesis and lymphangiogenesis, but not tumor growth. In contrast, expression of proVEGF-C obtained following mutation of the cleavage site (HSIIRR(227)SL to HSIISS(227)SL) inhibits angiogenesis and lymphangiogenesis as well as tumor growth. Our findings demonstrate the processing of proVEGF-C by PCs and highlight the potential use of PC inhibitors as agents for inhibiting malignancies induced by VEGF-C.

L22 ANSWER 5 OF 29 MEDLINE @ on STN
ACCESSION NUMBER: 1998167900 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9435294
TITLE: Avian VEGF-C: cloning, embryonic expression pattern and stimulation of the differentiation of VEGFR2-expressing endothelial cell precursors.
AUTHOR: Eichmann A
CORPORATE SOURCE: Institut d'Embryologie Cellulaire et Moleculaire du CNRS et du College de France, Nogent-sur-Marne, France.
eichmann@infobiogen.fr
AUTHOR: Corbel C; Jaffredo T; Breant C; Joukov V; Kumar V; Alitalo K; le Douarin N M
SOURCE: Development (Cambridge, England), (1998 Feb) Vol. 125, No.

4, pp. 743-52.
Journal code: 8701744. ISSN: 0950-1991. L-ISSN: 0950-1991.
DIGITAL OBJECT ID: <http://dx.doi.org/10.1242/dev.125.4.743>
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: MEDLINE; Priority Journals
FILE SEGMENT: Print
OTHER SOURCE: GENBANK-Y15837
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 10 Apr 1998
Last Updated on STN: 3 Mar 2000
Entered Medline: 2 Apr 1998
OS.CITING REF COUNT: 13 There are 13 MEDLINE records that cite this record
AB VEGF-C is a recently discovered secreted polypeptide related to the
angiogenic mitogen VEGF. We have isolated the quail VEGF-C cDNA and shown
that its protein product is secreted from transfected cells and
interacts with the avian VEGFR3 and VEGFR2. In situ hybridization shows
that quail VEGF-C mRNA is strongly expressed in regions destined to be
rich in lymphatic vessels, particularly the mesenteries, mesocardium and
myotome, in the region surrounding the jugular veins, and in the kidney.
These expression sites are similar to those observed in the mouse embryo
(E. Kukk, A. Lymboussaki, S. Taira, A. Kaipainen, M. Jeltsch, V.
Joukov and K. Alitalo, 1996, Development 122, 3829-3837). We have
observed VEGFR3-positive endothelial cells in proximity to most of the
VEGF-C-expressing sites, suggesting functional relationships between this
receptor-ligand couple. The comparison of the VEGF and VEGFR2 knockout
phenotypes had suggested the existence of another ligand for VEGFR2. We
therefore investigated the effect of VEGF-C on VEGFR2-positive cells
isolated from the posterior mesoderm of gastrulating embryos. We have
recently shown that VEGF binding triggers endothelial differentiation of
these cells, whereas hemopoietic differentiation appears to be mediated by
binding of a so far unidentified VEGFR2 ligand. We show here that VEGF-C
also triggers endothelial differentiation of these cells, presumably via
VEGFR2. These results indicate that VEGF and VEGF-C can act in a
redundant manner via VEGFR2. In conclusion, VEGF-C appears to act during
two different developmental phases, one early in posterior mesodermal
VEGFR2-positive endothelial cell precursors which are negative for VEGFR3
and one later in regions rich in lymphatic vessels at a time when
endothelial cells express both VEGFR2 and VEGFR3.

L22 ANSWER 6 OF 29 MEDLINE @ on STN
ACCESSION NUMBER: 1997164697 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9012504
TITLE: VEGF-C receptor binding and pattern of expression with
VEGFR-3 suggests a role in lymphatic vascular development.
AUTHOR: Kukk E
CORPORATE SOURCE: Molecular/Cancer Biology Laboratory, Haartman Institute,
University of Helsinki, Finland.
AUTHOR: Lymboussaki A; Taira S; Kaipainen A; Jeltsch M; Joukov V;
Alitalo K
SOURCE: Development (Cambridge, England), (1996 Dec) Vol. 122, No.
12, pp. 3829-37.
Journal code: 8701744. ISSN: 0950-1991. L-ISSN: 0950-1991.
DIGITAL OBJECT ID: <http://dx.doi.org/10.1242/dev.122.12.3829>
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: MEDLINE; Priority Journals
FILE SEGMENT: Print
OTHER SOURCE: GENBANK-U73620
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 6 Mar 1997
Last Updated on STN: 3 Mar 2000
Entered Medline: 24 Feb 1997
OS.CITING REF COUNT: 144 There are 144 MEDLINE records that cite this record
AB The vascular endothelial growth factor family has recently been expanded
by the isolation of two new VEGF-related factors, VEGF-B and VEGF-C. The
physiological functions of these factors are largely unknown. Here we

report the cloning and characterization of mouse VEGF-C, which is produced as a disulfide-linked dimer of 415 amino acid residue polypeptides, sharing an 85% identity with the human VEGF-C amino acid sequence. The recombinant mouse VEGF-C protein was secreted from transfected cells as VEGFR-3 (Flt4) binding polypeptides of 30-32x10(3) Mr and 22-23x10(3) Mr which preferentially stimulated the autophosphorylation of VEGFR-3 in comparison with VEGFR-2 (KDR). In situ hybridization, mouse VEGF-C mRNA expression was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins, such as the perimetonephric, axillary and jugular regions. In addition, the developing mesenterium, which is rich in lymphatic vessels, showed strong VEGF-C expression. VEGF-C was also highly expressed in adult mouse lung, heart and kidney, where VEGFR-3 was also prominent. The pattern of expression of VEGF-C in relation to its major receptor VEGFR-3 during the sprouting of the lymphatic endothelium in embryos suggests a paracrine mode of action and that one of the functions of VEGF-C may be in the regulation of angiogenesis of the lymphatic vasculature.

L22 ANSWER 7 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2022:2337221 CAPLUS Full-text
 DOCUMENT NUMBER: 181:125310
 TITLE: Insulin-like growth factor binding protein 7 promotes acute kidney injury by alleviating poly ADP ribose polymerase 1 degradation
 AUTHOR(S): Yu, Ju-tao; Hu, Xiao-wei; Yang, Qin; Shan, Run-run; Zhang, Yao; Dong, Ze-hui; Li, Hai-di; Wang, Jia-nan; Li, Chao; Xie, Shuai-shuai; Dong, Yu-hang; Ni, Wei-jian; Jiang, Ling; Liu, Xue-qi; Wei, Biao; Wen, Jia-gen; Liu, Ming-ming; Chen, Qi; Yang, Ya-ru; Zhang, Gui-yang; Zang, Hong-mei; Jin, Juan; Wu, Yong-gui; Zhong, Xiang; Li, Jun; Wang, Wei; Meng, Xiao-ming
 CORPORATE SOURCE: Inflammation and Immune Mediated Diseases Laboratory of Anhui Province, the Key Laboratory of Anti-inflammatory of Immune Medicines, Ministry of Education, Anhui Institute of Innovative Drugs, School of Pharmacy, Anhui Medical University, Hefei, Peop. Rep. China
 SOURCE: Kidney International (2022), 102(4), 828-844
 CODEN: KDYIA5; ISSN: 0085-2538
 DIGITAL OBJECT ID: 10.1016/j.kint.2022.05.026
 PUBLISHER: Elsevier Inc.
 DOCUMENT TYPE: Journal; (online computer file)
 LANGUAGE: English
 AB The novel biomarker, insulin-like growth factor binding protein 7 (IGFBP7), is used clin. to predict different types of acute kidney injury (AKI) and has drawn significant attention as a urinary biomarker. However, as a secreted protein in the circulation of patients with AKI, it is unclear whether IGFBP7 acts as a key regulator in AKI progression, and if mechanisms underlying its upregulation still need to be detd. Here we found that IGFBP7 is highly expressed in the blood and urine of patients and mice with AKI, possibly via a c-Jun-dependent mechanism, and is pos. correlated with kidney dysfunction. Global knockout of IGFBP7 ameliorated kidney dysfunction, inflammatory responses, and programmed cell death in murine models of cisplatin-, kidney ischemia/reperfusion-, and lipopolysaccharide-induced AKI. IGFBP7 mainly originated from kidney tubular epithelial cells. Conditional knockout of IGFBP7 from the kidney protected against AKI. By contrast, rescue of IGFBP7 expression in IGFBP7-knockout mice restored kidney damage and inflammation. IGFBP7 function was detd. in vitro using recombinant IGFBP7 protein, IGFBP7 knockdown, or overexpression. Addnl., IGFBP7 was found to bind to poly [ADP-ribose] polymerase 1 (PARP1) and inhibit its degrdn. by antagonizing the E3 ubiquitin ligase ring finger protein 4 (RNF4). Thus, IGFBP7 in circulation acts as a biomarker and key mediator of AKI by inhibiting RNF4/PARP1-mediated tubular injury and inflammation. Hence, over-activation of the IGFBP7/PARP1 axis represents a promising target for AKI treatment.
 OS.CITING REF COUNT: 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)
 REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 8 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2022:934917 CAPLUS Full-text
 DOCUMENT NUMBER: 180:155468
 TITLE: Proteomic analysis reveals exercise training induced remodelling of hepatokine secretion and uncovers syndecan-4 as a regulator of hepatic lipid metabolism
 AUTHOR(S): De Nardo, William; Miotto, Paula M.; Bayliss, Jacqueline; Nie, Shuai; Keenan, Stacey N.; Montgomery, Magdalene K.; Watt, Matthew J.
 CORPORATE SOURCE: Department of Anatomy and Physiology, School of Biomedical Sciences, Faculty of Medicine, Dentistry & Health Sciences, The University of Melbourne, VIC, 3010, Australia
 SOURCE: Molecular Metabolism (2022), 60, 101491
 CODEN: MMOEAS; ISSN: 2212-8778
 DIGITAL OBJECT ID: 10.1016/j.molmet.2022.101491
 PUBLISHER: Elsevier GmbH
 DOCUMENT TYPE: Journal; (online computer file)
 LANGUAGE: English

AB Non-alc. fatty liver disease (NAFLD) is linked to impaired lipid metab. and systemic insulin resistance, which is partly mediated by altered secretion of liver proteins known as hepatokines. Regular phys. activity can resolve NAFLD and improve its metabolic comorbidities, however, the effects of exercise training on hepatokine secretion and the metabolic impact of exercise-regulated hepatokines in NAFLD remain unresolved. Herein, we examd. the effect of endurance exercise training on hepatocyte secreted proteins with the aim of identifying proteins that regulate metab. and reduce NAFLD severity. C57BL/6 mice were fed a high-fat diet for six weeks to induce NAFLD. Mice were exercise trained for a further six weeks, while the control group remained sedentary. Hepatocytes were isolated two days after the last exercise bout, and intracellular and secreted proteins were detected using label-free mass spectrometry. Hepatocyte secreted factors were applied to skeletal muscle and liver ex vivo and insulin action and fatty acid metab. were assessed. Syndecan-4 (SDC4), identified as an exercise-responsive hepatokine, was overexpressed in the livers of mice using adeno-assocd. virus. Whole-body energy homeostasis was assessed by indirect calorimetry and skeletal muscle and liver metab. was assessed using radiometric techniques. Proteomics anal. detected 2657 intracellular and 1593 secreted proteins from mouse hepatocytes. Exercise training remodelled the hepatocyte proteome, with differences in 137 intracellular and 35 secreted proteins. Bioinformatic anal. of hepatocyte secreted proteins revealed enrichment of tumor suppressive proteins and proteins involved in lipid metab. and mitochondrial function, and suppression of oncogenes and regulators of oxidative stress. Hepatocyte secreted factors from exercise trained mice improved insulin action in skeletal muscle and increased hepatic fatty acid oxidn. Hepatocyte-specific overexpression of SDC4 reduced hepatic steatosis, which was assocd. with reduced hepatic fatty acid uptake, and blunted pro-inflammatory and pro-fibrotic gene expression. Treating hepatocytes with recombinant ectodomain of SDC4 (secreted form) recapitulated these effects with reduced fatty acid uptake, lipid storage and lipid droplet accumulation. Remodelling of hepatokine secretion is an adaptation to regular exercise training that induces changes in metab. in the liver and skeletal muscle. SDC4 is a novel exercise-responsive hepatokine that decreases fatty acid uptake and reduces steatosis in the liver. By understanding the proteomic changes in hepatocytes with exercise, these findings have potential for the discovery of new therapeutic targets for NAFLD.

OS.CITING REF COUNT: 4 THERE ARE 4 CAPLUS RECORDS THAT CITE THIS RECORD (4 CITINGS)
 REFERENCE COUNT: 85 THERE ARE 85 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2022:792140 CAPLUS Full-text
 DOCUMENT NUMBER: 183:290217
 TITLE: Harnessing secretory pathway differences between HEX293 and CHO to rescue production of difficult to express proteins
 AUTHOR(S): Malm, Magdalena; Kuo, Chih-Chung; Barzadd, Mona Moradi; Mebrahtu, Aman; Wistbacka, Num; Razavi, Ronia;

Volk, Anna-Luisa; Lundqvist, Magnus; Kotol, David;
Tegel, Hanna; Hober, Sophia; Edfors, Fredrik;
Graeslund, Torbjørn; Chotteau, Veronique; Field, Ray;
Varley, Paul G.; Roth, Robert G.; Lewis, Nathan E.;
Hatton, Diane; Rockberg, Johan

CORPORATE SOURCE: Dept. of Protein Science, KTH - Royal Institute of
Technology, Stockholm, SE-106 91, Swed.

SOURCE: Metabolic Engineering (2022), 72, 171-187
CODEN: MEENFM; ISSN: 1096-7176

DIGITAL OBJECT ID: 10.1016/j.ymben.2022.03.009

PUBLISHER: Elsevier B.V.

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English

AB Biologics represent the fastest growing group of therapeutics, but many
advanced recombinant protein moieties remain difficult to produce.
Here, we identify metabolic engineering targets limiting expression of
recombinant human proteins through a systems biol. anal. of the
transcriptomes of CHO and HEK293 during recombinant expression. In
an expression comparison of 24 difficult to express proteins, one third of
the challenging human proteins displayed improved secretion upon host
cell swapping from CHO to HEK293. Guided by a comprehensive
transcriptomics comparison between cell lines, esp. highlighting
differences in secretory pathway utilization, a co-expression screening
of 21 secretory pathway components validated ATF4, SRP9, JUN, PDIA3 and
HSPA8 as productivity boosters in CHO. Moreover, more heavily
glycosylated products benefitted more from the elevated activities of the
N- and O-glycosyltransferases found in HEK293. Collectively, our
results demonstrate the utilization of HEK293 for expression rescue of
human proteins and suggest a methodol. for identification of secretory
pathway components for metabolic engineering of HEK293 and CHO.

OS.CITING REF COUNT: 8 THERE ARE 8 CAPLUS RECORDS THAT CITE THIS RECORD
(8 CITINGS)

REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 10 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2020:2599335 CAPLUS Full-text

DOCUMENT NUMBER: 174:535456

TITLE: Lack of evidence for expression and function of IL-39
in human immune cells

AUTHOR(S): Ecoeur, Florence; Weiss, Jessica; Schleege, Simone;
Guntermann, Christine

CORPORATE SOURCE: Autoimmunity, Transplantation and Inflammation,
Novartis Institutes for BioMedical Research, Switz.

SOURCE: PLoS One (2020), 15(12), e0242329
CODEN: POLNCL; ISSN: 1932-6203
URL: <https://journals.plos.org/plosone/>

DIGITAL OBJECT ID: 10.1371/journal.pone.0242329

PUBLISHER: Public Library of Science

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English

AB Members of the IL-6/IL-12 cytokine family are crit. regulators of innate
and adaptive immunity and have emerged as key players controlling
inflammatory and autoimmune disorders. This cytokine family comprises of
IL-12, IL-23, IL-27, and IL-35, each consisting of distinct α - and
 β -cytokine subunits that form heterodimers. A new member of this
family, IL-39, was identified in the murine species and was shown to
consist of the IL-23p19 and Epstein-Barr Virus-induced 3 (EBI3) subunits.
Subsequently, it was shown that IL-39 was implicated in the
immunopathogenesis of murine exptl. lupus erythematosus. The existence of
IL-39 in the human system has yet to be confirmed. Based on the clin.
success of IL-23p19 neutralizing approaches in moderate-to-severe
psoriasis, anti-IL-23p19 antibodies in the clinic may not only neutralize
IL-23, but addnl. IL-39, implying that IL-39 might also contribute to the
pathogenesis of psoriasis. It is therefore pivotal to demonstrate IL-39
expression and to characterize its function in the human system. In this
study, we provided evidence for the existence of secreted heterodimeric
p19 and EBI3 complexes in supernatants originating from p19 and EBI3
transfected HEK293FT cells. We attempted to detect IL-39 expression
from stimulated human primary B cells, human keratinocytes and in vitro
polarized human macrophages. Whereas, the expression of p19 and EBI3 mRNA
was elevated, we failed to detect p19 and EBI3 heterodimers. Functional

assays were conducted with conditioned media contg. human IL-39 or with a human recombinant IL-39 Fc protein. Immune cells targeted by IL-39 in mouse, such as neutrophils and PBMCs, did not respond to human IL-39 stimulation and IL-39 failed to activate STAT3 in a reporter cell line. These results suggest that, while the secretion of p19/EBI3 complexes can be forced in human cells, it is secreted below the lower quantity of detection or it has no functional role.

OS.CITING REF COUNT: 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2020:1787863 CAPLUS Full-text

DOCUMENT NUMBER: 174:397724

TITLE: Transforming growth factor beta (TGF- β) is activated by the CtBP2-p300-AP1 transcriptional complex in chronic renal failure

AUTHOR(S): Zhou, Ping; Wan, Xiaoxiao; Zou, Yan; Chen, Zhi; Zhong, Aimin

CORPORATE SOURCE: Department of Nephrology, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, Jiangxi, 330006, Peop. Rep. China

SOURCE: International Journal of Biological Sciences (2020), 16(2), 204-215

CODEN: IJBSB9; ISSN: 1449-2288

URL: <http://www.biolsci.org>

DIGITAL OBJECT ID: 10.7150/ijbs.38841

PUBLISHER: Ivyspring International Publisher

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English

AB Chronic renal failure (CRF), also known as chronic kidney disease (CKD), is a common renal disorder characterized by gradual kidney dysfunction. Mol. dissection reveals that transforming growth factor beta (TGF- β) plays a central role in the pathogenesis of CRF. However, the mechanism underlying TGF- β upregulation has not been demonstrated. Here, we verified that the elevated level of TGF- β was assocd. with the severity of CRF stages and the activation of TGF- β -mediated signaling in 120 renal biopsies from CRF patients. By analyzing the promoter region of the TGFB1 gene, we identified one AP-1 (activator protein 1) and four NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) binding sites. Knockdown of two AP-1 subunits (c-Jun and c-FOS) or blockage of AP-1 signaling with two inhibitors T-5224 and SR11302 could cause the downregulation of TGFB1, whereas knockdown of two NF- κ B subunits (p65 and p50) or blockage of NF- κ B signaling with two inhibitors TPCA1 and BOT-64 could not change the expression of TGFB1. Using mass spectrometry and coimmunopptn. analyses, we found that both c-Jun and c-FOS formed a complex with CtBP2 (C-terminal binding protein 2) and histone acetyltransferase p300. Our in vitro data demonstrated that induction of CtBP2 by recombinant IL-1 β (interleukin-1 beta) led to the upregulation of TGFB1 and the activation of TGF- β downstream signaling, while knockdown of CtBP2 resulted in the reversed effects. Using chromatin immunopptn. assays, we revealed that the CtBP2-p300-AP1 complex specifically bound to the promoter of TGFB and that knockdown or blockage of CtBP2 significantly decreased the occupancies of the p300 and AP-1 subunits. Our results support a model in which the CtBP2-p300-AP1 transcriptional complex activates the expression of TGFB1, increasing its prodn. and extracellular secretion. The secreted TGF- β binds to its receptors and initiates downstream signaling.

OS.CITING REF COUNT: 19 THERE ARE 19 CAPLUS RECORDS THAT CITE THIS RECORD (19 CITINGS)

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2016:1045636 CAPLUS Full-text

DOCUMENT NUMBER: 167:209204

TITLE: Antagonizing canonical Wnt signaling pathway by recombinant human sFRP4 purified from E. coli and its implications in cancer therapy

AUTHOR(S): Ghoshal, Archita; Ghosh, Siddhartha Sankar

CORPORATE SOURCE: Department of Biosciences and Bioengineering,

Guwahati, Indian Institute of Technology Guwahati, 39,
India
SOURCE: Molecular and Cellular Biochemistry (2016), 418(1-2),
119-135
CODEN: MCBIB8; ISSN: 0300-8177
DIGITAL OBJECT ID: 10.1007/s11010-016-2738-6
PUBLISHER: Springer
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English

AB The Wnt signaling pathway plays a predominant role in aberrant proliferation in myriad of cancers. In non-cancerous cells, Wnts are blocked by the secreted frizzled-related proteins (sFRPs) that are generally downregulated in cancer cells. We have purified and characterized bacterially expressed glutathione S-transferase-tagged SFRP4 from a novel clone generated from human cell origin. Cervical cancer (HeLa) and lung cancer (A549) cells, in which Wnt and assocd. genes were found to be expressed, were treated with the purified recombinant sFRP4, which revealed a significant dose-dependent cell growth inhibition up to 40 %. The current investigation on functionality of this bacterially produced recombinant sFRP4 in arresting cancer cell proliferation is the first of its kind, where G2/M phase arrest and early apoptosis were evident. Increase in phosphorylated β -catenin in sFRP4 treatment indicated inhibition of Wnt pathway, which was further confirmed by downregulation of pro-proliferative genes, namely cyclin D1, c-myc, and survivin. Functional activity of recombinant sFRP4 was further exploited in co-therapy module with chemotherapeutic drugs to decipher mol. events. Collectively, our study on purified recombinant sFRP4 from bacterial host holds great promise in targeting Wnt signaling for exploring new strategies to combat cancer.

OS.CITING REF COUNT: 10 THERE ARE 10 CAPLUS RECORDS THAT CITE THIS RECORD (10 CITINGS)
REFERENCE COUNT: 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2016:823705 CAPLUS Full-text
DOCUMENT NUMBER: 165:4755
TITLE: Single chain antibody against β -site of amyloid precursor protein inhibits the generation of β -amyloid peptide
AUTHOR(S): Huang, Wei; Zhou, Lirong; Li, Xiao-ou; Sun, Hongxia; Zhou, Ting; Huang, Xiaogang; Liu, Qiao
CORPORATE SOURCE: Department of Preclinical Medicine, Wuhan Institute of Medical Sciences, Wuhan, Hubei, 430014, Peop. Rep. China
SOURCE: Xibao Yu Fenzi Mianyixue Zazhi (2015), 31(10), 1408-1412
CODEN: XFMZFM; ISSN: 1007-8738
PUBLISHER: Xibao Yu Fenzi Mianyixue Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Objective To construct and express a single chain fragment of variety region (scFv) against β -site of amyloid precursor protein (APP), and evaluate the effect of scFv on α - and β -processing of APP. Methods The β -site specific scFv 2H10 was amplified and fused with signal sequence Ig κ and myc tag by PCR to create the expression cassette, which was then subcloned into expression vector pcDNA3.1hyg(+). The plasmid was transferred into CHO cells over-expressing Swedish mutation type human APP695 (APP695 sw/CHO). The pos. clones were identified by hygromycin B. The concns. of A β 40 and sol. APP α (sAPP α) in the conditioned media supernatant were measured by sandwich ELISA. Results The recombinant eukaryotic expression plasmids were identified by PCR, restriction endonuclease digestion and DNA sequencing. The expression of s.c. Fv was confirmed by Western blotting. When scFv 2H10 was stably expressed in APP695 sw/CHO, the extracellular level of A β 40 decreased by 30.4%, while sAPP α increased by 23.1%. Conclusion The APP β -site scFv 2H10 could inhibit the secretion of A β . Meanwhile, it might be favorable for the α -processing of APP. Hence it could be explored as an inhibitor of β -site APP-cleaving enzyme (BACE).

L22 ANSWER 14 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2015:1197464 CAPLUS Full-text
 DOCUMENT NUMBER: 164:424354
 TITLE: NELL-1 in the treatment of osteoporotic bone loss
 AUTHOR(S): James, Aaron W.; Shen, Jia; Zhang, Xinli; Asatrian, Greg; Goyal, Raghav; Kwak, Jin H.; Jiang, Lin; Bengs, Benjamin; Culiati, Cymbeline T.; Turner, A. Simon; Seim, Howard B., III; Wu, Benjamin M.; Lyons, Karen; Adams, John S.; Ting, Kang; Soo, Chia
 CORPORATE SOURCE: Department of Orthopaedic Surgery and the Orthopaedic Hospital Research Center, UCLA and Orthopaedic Hospital, University of California, Los Angeles, CA, 90095, USA
 SOURCE: Nature Communications (2015), 6, 7362
 CODEN: NCAOBW; ISSN: 2041-1723
 DIGITAL OBJECT ID: 10.1038/ncomms8362
 PUBLISHER: Nature Publishing Group
 DOCUMENT TYPE: Journal; (online computer file)
 LANGUAGE: English
 AB NELL-1 is a secreted, osteoinductive protein whose expression rheostatically controls skeletal ossification. Overexpression of NELL-1 results in craniosynostosis in humans and mice, whereas lack of Nell-1 expression is assocd. with skeletal undermineralization. Here we show that Nell-1-haploinsufficient mice have normal skeletal development but undergo age-related osteoporosis, characterized by a redn. in osteoblast:osteoclast (OB:OC) ratio and increased bone fragility. Recombinant NELL-1 binds to integrin $\beta 1$ and consequently induces Wnt/ β -catenin signalling, assocd. with increased OB differentiation and inhibition of OC-directed bone resorption. Systemic delivery of NELL-1 to mice with gonadectomy-induced osteoporosis results in improved bone mineral d. When extended to a large animal model, local delivery of NELL-1 to osteoporotic sheep spine leads to significant increase in bone formation. Altogether, these findings suggest that NELL-1 deficiency plays a role in osteoporosis and demonstrate the potential utility of NELL-1 as a combination anabolic/antiosteoclastic therapeutic for bone loss.
 OS.CITING REF COUNT: 60 THERE ARE 60 CAPLUS RECORDS THAT CITE THIS RECORD (60 CITINGS)
 REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
 L22 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN
 ACCESSION NUMBER: 2015:439888 CAPLUS Full-text
 DOCUMENT NUMBER: 163:202165
 TITLE: Cardiac myocyte-derived follistatin-like 1 prevents renal injury in a subtotal nephrectomy model
 AUTHOR(S): Hayakawa, Satoko; Ohashi, Koji; Shibata, Rei; Kataoka, Yoshiyuki; Miyabe, Megumi; Enomoto, Takashi; Joki, Yusuke; Shimizu, Yuuki; Kambara, Takahiro; Uemura, Yusuke; Yuasa, Daisuke; Ogawa, Hayato; Matsuo, Kazuhiro; Hiramatsu-Ito, Mizuho; van den Hoff, Maurice J. B.; Walsh, Kenneth; Murohara, Toyooki; Ouchi, Noriyuki
 CORPORATE SOURCE: Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
 SOURCE: Journal of the American Society of Nephrology (2015), 26(3), 636-646
 CODEN: JASNEU; ISSN: 1046-6673
 DIGITAL OBJECT ID: 10.1681/ASN.2014020210
 PUBLISHER: American Society of Nephrology
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Heart disease contributes to the progression of CKD. Heart tissue produces a no. of secreted proteins, also known as cardiokines, which participate in intercellular and intertissue communication. We recently reported that follistatin-like 1 (Fstl1) functions as a cardiokine with cardioprotective properties. Here, we investigated the role of cardiac Fstl1 in renal injury after subtotal nephrectomy. Cardiac-specific Fstl1-deficient (cFstl1-KO) mice and wild-type mice were subjected to subtotal (5/6) nephrectomy. CFstl1-KO mice showed exacerbation of urinary albumin excretion, glomerular hypertrophy, and tubulointerstitial fibrosis after subtotal renal ablation compared with wild-type mice. CFstl1-KO mice also exhibited increased mRNA levels of proinflammatory

cytokines, including TNF- α and IL-6, NADPH oxidase components, and fibrotic mediators, in the remnant kidney. Conversely, systemic administration of adenoviral vectors expressing Fstl1 (Ad-Fstl1) to wild-type mice with subtotal nephrectomy led to amelioration of albuminuria, glomerular hypertrophy, and tubulointerstitial fibrosis, accompanied by reduced expression of proinflammatory mediators, NADPH oxidase components, and fibrotic markers in the remnant kidney. In cultured human mesangial cells, treatment with recombinant FSTL1 attenuated TNF- α -stimulated expression of proinflammatory cytokines. Treatment of mesangial cells with FSTL1 augmented the phosphorylation of AMP-activated protein kinase (AMPK), and inhibition of AMPK activation abrogated the anti-inflammatory effects of FSTL1. These data suggest that Fstl1 functions in cardiorenal communication and that the lack of Fstl1 prodn. by myocytes promotes glomerular and tubulointerstitial damage in the kidney.

OS.CITING REF COUNT: 35 THERE ARE 35 CAPLUS RECORDS THAT CITE THIS RECORD (35 CITINGS)
 REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 16 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2014:1694970 CAPLUS Full-text

DOCUMENT NUMBER: 162:48099

TITLE: Green fluorescent protein fused to peptide agonists of two dissimilar G protein-coupled receptors: novel ligands of the bradykinin B2 (rhodopsin family) receptor and parathyroid hormone PTH1 (secretin family) receptor

AUTHOR(S): Charest-Morin, Xavier; Fortin, Jean-Philippe; Bawolak,

Marié-Thérèse; Lodge, Robert; Marceau, François

CORPORATE SOURCE: Centre de Recherche en Rhumatologie et Immunologie, CHU de Québec, Université Laval, QC, G1V 4G2, Can.

SOURCE: Pharmacology Research & Perspectives (2013), 1(1),

e00004/1-e00004/13

CODEN: PRPHCA; ISSN: 2052-1707

DIGITAL OBJECT ID: 10.1002/prp2.4

PUBLISHER: John Wiley & Sons Ltd.

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English

AB We hypothesized that peptide hormone sequences that stimulate and internalize G protein-coupled receptors (GPCRs) could be prolonged with a functional protein cargo. To verify this, we have selected two widely different pairs of peptide hormones and GPCRs that nevertheless share agonist-induced arrestin-mediated internalization. For the parathyroid hormone (PTH) PTH1 receptor (PTH1R) and the bradykinin (BK) B2 receptor (B2R), we have designed fusion proteins of the agonists PTH1-34 and maximakinin (MK, a BK homolog) with the enhanced green fluorescent protein (EGFP), thus producing candidate high mol. wt. ligands. According to docking models of each hormone to its receptor, EGFP was fused either at the N-terminus (MK) or C-terminus (PTH1-34) of the ligand; the last construction is also secreted due to inclusion of the preproinsulin signal peptide and has been produced as a conditioned medium. EGFP-MK has been produced as a lysate of transfected cells. Using an ELISA (ELISA) for GFP, av. concns. of 1.5 and 1670 nmol/L, resp., of ligand were found in these preps. The functional properties and potential of these analogs for imaging receptor-expressing cells were examd. Microscopic and cytofluorometric evidence of specific binding and internalization of both fusion proteins was obtained using recipient HEK 293a cells that expressed the cognate recombinant receptor. Endosomal colocalization studies were conducted (Rab5, Rab7, β -arrestin1). Evidence of agonist signaling was obtained (expression of c-Fos, cAMP responsive element (CRE) reporter gene for PTH1-34-EGFP). The constructs PTH1-34-EGFP and EGFP-MK represent bona fide agonists that support the feasibility of transporting protein cargoes inside cells using GPCRs.

OS.CITING REF COUNT: 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 17 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2014:1376605 CAPLUS Full-text

DOCUMENT NUMBER: 162:88935

TITLE: A tagged parathyroid hormone derivative as a carrier of antibody cargoes transported by the G protein coupled PTH1 receptor
AUTHOR(S): Charest-Morin, Xavier; Fortin, Jean-Philippe; Lodge, Robert; Allaeys, Isabelle; Poubelle, Patrice E.; Marceau, Francois
CORPORATE SOURCE: Centre de recherche en Rhumatologie et Immunologie, Quebec, QC, G1V 4G2, Can.
SOURCE: Peptides (New York, NY, United States) (2014), 60, 71-79
DIGITAL OBJECT ID: CODEN: PPTDD5; ISSN: 0196-9781 10.1016/j.peptides.2014.08.001
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English

AB Based on the known fact that the parathyroid hormone (PTH) might be extended at its C-terminus with biotechnol. protein cargoes, a vector directing the secretion of PTH1-84 C-terminally fused with the antigenic epitope myc (PTH-myc) was exploited. The functional properties and potential of this analog for imaging PTH1R-expressing cells were examd. The PTH-myc construct was recombinantly produced as a conditioned medium (CM) of transfected HEK 293a cells (typical concns. of 187 nM estd. with ELISAs for PTH). PTH-myc CM induced cAMP formations (10 min), with a minor loss of potency relative to authentic PTH1-84, and c-Fos expression (1-3 h). Treatment of recipient HEK 293a cells transiently expressing PTH1R with PTH-myc CM (supplemented with a fluorescent monoclonal anti-myc tag antibody, either 4A6 or 9E10) allowed the labeling of endosomal structures pos. for Rab5 and/or for β -arrestin1 (microscopy, cytofluorometry). Authentic PTH was inactive in this respect, ruling out a non-specific form of endocytosis like pinocytosis. Using a horseradish peroxidase-conjugated secondary antibody, the endocytosis of the PTH-myc-based antibody complex by endogenous PTH1R was evidenced in MG-63 osteoblastoid cells. The secreted construct PTH-myc represents a bona fide agonist that supports the feasibility of transporting cargoes of considerable mol. wt. inside cells using arrestin and Rab5-mediated PTH1R endocytosis. PTH-myc is also transported into cells that express PTH1R at a physiol. level. Such tagged peptide hormones may be part of a cancer chemotherapy scheme exploiting a modular cytotoxic secondary antibody and the receptor repertoire expressed in a given tumor.

OS.CITING REF COUNT: 5 THERE ARE 5 CAPLUS RECORDS THAT CITE THIS RECORD (5 CITINGS)
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 18 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN
ACCESSION NUMBER: 2013:928635 CAPLUS Full-text
DOCUMENT NUMBER: 159:159099
TITLE: Identification of autocrine growth factors secreted by CHO cells for applications in single-cell cloning media
AUTHOR(S): Lim, U. Ming; Yap, Miranda Gek Sim; Lim, Yoon Pin; Goh, Lin-Tang; Ng, Say Kong
CORPORATE SOURCE: Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), Singapore, 138668, Singapore
SOURCE: Journal of Proteome Research (2013), 12(7), 3496-3510
DIGITAL OBJECT ID: CODEN: JPROBS; ISSN: 1535-3893 10.1021/pr400352n
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English

AB Chinese hamster ovary (CHO) cell lines are widely used for the expression of therapeutic recombinant proteins, including monoclonal antibodies and other biologics. For manufg., cells derived from a single-cell clone are typically used to ensure product consistency. Presently, fetal bovine serum (FBS) is commonly used to support low cell d. cultures to obtain clonal cell populations because cells grow slowly, or even do not survive at low cell densities in protein-free media. However, regulatory authorities have discouraged the use of FBS to reduce the risk of contamination by adventitious agents from animal-derived components. In this study, we demonstrated how a complementary mass spectrometry-based shotgun proteomics strategy enabled the identification

of autocrine growth factors in CHO cell-conditioned media, which has led to the development of a fully defined single-cell cloning media that is serum and animal component-free. Out of 290 ~~secreted~~ proteins that were identified, eight ~~secreted~~ growth factors were reported for the first time from CHO cell cultures. By supplementing a combination of these growth factors to protein-free basal media, single cell growth of CHO cells was improved with cloning efficiencies of up to 30%, a 2-fold improvement compared to un-supplemented basal media. Complementary effects of these autocrine growth factors with other paracrine growth factors were also demonstrated when the mixt. improved cloning efficiency to 42%, similar to that for the conditioned medium.

OS.CITING REF COUNT: 35 THERE ARE 35 CAPLUS RECORDS THAT CITE THIS RECORD (35 CITINGS)
 REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2010:59732 CAPLUS Full-text

DOCUMENT NUMBER: 153:610938

TITLE: VEGFR-3 gene transfection for its soluble VEGFR-3

release in lymphatic endothelial progenitor cells

AUTHOR(S): Ao, Hong; Tan, Yu-zhen; Wang, Hai-jie; Guo, Hai-dong

CORPORATE SOURCE: Department of Anatomy and Histology and Embryology, Shanghai Medical School, Fudan University, Shanghai, 200032, Peop. Rep. China

SOURCE: Zhongguo Shiyao Dongwu Xuebao (2009), 17(4), 252-257, 1 plate

CODEN: ZSDXCD; ISSN: 1005-4847

PUBLISHER: Zhongguo Shiyao Dongwu Xuehui

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB Vascular endothelial growth factor receptor 3 (VEGFR-3), a specific receptor of vascular endothelial growth factor C (VEGF-C), is a new member of tyrosine kinase receptor family. VEGFR-3 is consisted of extracellular, transmembrane and intracellular regions. The extracellular region comprises of seven Ig-like domains, among which the first three domains (1-3Ig) play esp. important roles in VEGF-C/VEGFR-3 signaling pathway, because these domains are responsible for the binding between VEGFR-3 and VEGF-C. Lymphatic endothelial progenitor cells (LEPCs) are mainly localized to the bone marrow, but can be released from bone marrow into diseased tissues in case of inflammation, ischemia, tumor, etc., and differentiated into lymphatic endothelial cells (LEC) to participate in the lymphangiogenesis in tumor tissues. As VEGF-C/VEGFR-3 signaling pathway is crit. for the differentiation of LEPCs into LEC in tumor tissue, blockage of this signaling pathway will therefore be useful for inhibiting lymphangiogenesis and for the anti-tumor treatment. It is suggested that sol. VEGFR-3 can interfere with the binding between the VEGF-C and membrane-bound VEGFR-3, and thus can block VEGF-C/VEGFR-3 signaling pathway. In this study, we investigate the ~~secretion~~ of sol. VEGFR-3 proteins in LEPCs after transfected with recombinant adenovirus contg. VEGFR-3 (1-3Ig) gene of BABL/c mouse and the biol. activity of the ~~secreted~~ protein. The gene encoding VEGFR-3 (1-3Ig) was obtained through nested RT-PCR from BABL/c mouse placenta, and human CD33 signal peptide was added to the N-terminal of the PCR product through recombinant PCR technique. Then the gene fragment was cloned into the adenovirus shuttle plasmid pDC316-IRES-EGFP. After confirmed by restriction enzyme digestion and DNA sequencing, the recombinant adenovirus vector carrying VEGFR-3 (1-3Ig) was transfected into 293-cells together with the packing vector to produce the recombinant adenovirus. The recombinant adenovirus was used to transfect LEPCs, and ELISA was employed to quantify the amt. of sol. VEGFR-3 protein in the culture medium from transfected LEPCs. The biol. activity of the sol. VEGFR-3 was detd. by its ability to neutralize VEGF-C. Results showed that an adenovirus expression vector for sol. VEGFR-3 was successfully constructed, which can produce high titer recombinant adenovirus contg. VEGFR-3 (1-3Ig) of BABL/c mouse in 293-cells after transfection with packing vector. On transfection with the recombinant adenovirus carrying VEGFR-3 (1-3Ig), LEPCs can release sol. VEGFR-3 as detd. by ELISA, and the released VEGFR-3 can neutralize VEGFC efficiently. In conclusion, we demonstrated that LEPCs can express and ~~secrete~~ sol. VEGFR-3 protein after transfected with recombinant adenovirus contg. BABL/c mouse VEGFR-3 (1-3Ig) gene, and the released sol. VEGFR-3 protein

to neutralize VEGF-C. This study has laid a foundation for the application of VEGFR-3 gene-transfected LEPCs in the antitumor treatment.

L22 ANSWER 20 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2009:976726 CAPLUS Full-text
DOCUMENT NUMBER: 151:261260
TITLE: Recombinant bacteria with Escherichia coli hemolysin secretion system and increased expression and/or secretion of HlyA and their manufacture and uses
INVENTOR(S): Hotz, Christian; Gentschev, Ivaylo; Rapp, Ulf R.; Goebel, Werner; Fensterle, Joachim
PATENT ASSIGNEE(S): Aeterna Zentaris GmbH, Germany
ULTIMATE OWNER: AETERNA ZENTARIS, INC
ULTIMATE OWNER STANDARD: Aeterna Zentaris
SOURCE: PCT Int. Appl., 58pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2009098246	A1	20090813	WO 2009-EP51296	20090205
CA 2714276	A1	20090813	CA 2009-2714276	20090205
US 20090208461	A1	20090820	US 2009-365944	20090205
AR 70568	A1	20100421	AR 2009-100387	20090205
EP 2254902	A1	20101201	EP 2009-709046	20090205
MX 2010008555	A	20100830	MX 2010-8555	20100804
PRIORITY APPLN. INFO.:			EP 2008-101280	A 20080205
			US 2008-26158P	P 20080205
			WO 2009-EP51296	W 20090205

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2009098246	A1	Dead	20201202
CA 2714276	A1	Dead	20201120
US 20090208461	A1	Dead	20201121
AR 70568	A1	Alive	20201120
EP 2254902	A1	Dead	20201202
MX 2010008555	A	Alive	20201120

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB Recombinant Salmonella typhi Ty21a bacteria are provided with the Escherichia coli hemolysin secretion system and increased expression and/or increased secretion of full-length or partial HlyA. This transport machinery is the prototype type I secretion system (T1SS) and consists of three different components, namely HlyB, HlyD, and TolC; the fusion of HlyA to the C-terminus of heterologous antigens leads to efficient secretion of proteins by the recombinant bacteria. The recombinant bacteria may further comprise rfaH and/or rpoN genes integrated into the bacterial chromosome or, preferably, located on a plasmid, and the rpoS gene is deleted or inactivated. Attenuation is caused by deletion or inactivation of at least one gene selected from the group consisting of araA, aro, asd, gal, pur, cya, crp, phoP/Q, omp. The rfaH-Ty21a secretes heterologous antigens via T1SS and allows vaccination against both the carrier antigen and typhoid fever. Also disclosed are a process of manufg. thereof and uses of such bacterium as a medicament.

OS.CITING REF COUNT: 2 THERE ARE 2 CAPLUS RECORDS THAT CITE THIS RECORD (2 CITINGS)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
AR 70568 A1	AR 2009-100387	Register
CA 2714276 A1	CA 2009-2714276	Register
EP 2254902 A1	EP 2009-709046	Register Federated Reg.
MX 2010008555 A	MX 2010-8555	Register
US 20090208461 A1	US 2009-365944	Register Global Dossier
WO 2009098246 A1	WO 2009-EP51296	Register Global Dossier

L22 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2008:588852 CAPLUS Full-text
 DOCUMENT NUMBER: 148:536017
 TITLE: Attenuated bacteria expressing recombinant antigens and protein toxins and their use in tumor vaccines and immunotherapy
 INVENTOR(S): Fensterle, Joachim; Gentschev, Ivaylo; Rapp, Ulf R.; Goebel, Werner
 PATENT ASSIGNEE(S): Aeterna Zentaris G.m.b.H., Germany
 ULTIMATE OWNER: AETERNA ZENTARIS, INC
 ULTIMATE OWNER STANDARD: Aeterna Zentaris
 SOURCE: Eur. Pat. Appl., 86pp.
 CODEN: EPXXDW
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1921149	A1	20080514	EP 2006-123974	20061113
AU 2007321266	A1	20080522	AU 2007-321266	20071113
AU 2007321266	B2	20121115		
CA 2669219	A1	20080522	CA 2007-2669219	20071113
WO 2008058944	A1	20080522	WO 2007-EP62237	20071113
AR 63795	A1	20090218	AR 2007-105040	20071113
EP 2092067	A1	20090826	EP 2007-822513	20071113
EP 2092067	B1	20100602		
KR 2009092803	A	20090901	KR 2009-7012089	20071113
KR 1505413	B1	20150325		
CN 101595219	A	20091202	CN 2007-80044576	20071113
CN 101595219	B	20160127		
JP 2010508861	T	20100325	JP 2009-536716	20071113
JP 5479102	B2	20140423		
AT 469971	T	20100615	AT 2007-822513	20071113
PT 2092067	E	20100831	PT 2007-822513	20071113
ES 2347019	T3	20101022	ES 2007-822513	20071113
ME 1846	B	20110228	ME 2010-387	20071113
US 20110287037	A1	20111124	US 2007-939254	20071113
US 8669091	B2	20140311		
NZ 576947	A	20111222	NZ 2007-576947	20071113
RU 2447145	C2	20120410	RU 2009-122560	20071113
IL 198736	A	20120830	IL 2007-198736	20071113
TW I394834	B	20130501	TW 2007-142857	20071113
BR 2007019003	A2	20131217	BR 2007-19003	20071113
ZA 2009003022	A	20100331	ZA 2009-3022	20090504
PH 12009500878	B1	20170111	PH 2009-500878	20090506
IN 2009KN01749	A	20090612	IN 2009-KN1749	20090511
MX 2009005038	A	20090525	MX 2009-5038	20090512
NO 2009002278	A	20090813	NO 2009-2278	20090612
HK 1138314	A1	20161007	HK 2010-100634	20100121
CY 1111035	T1	20150611	CY 2010-1100767	20100819
PRIORITY APPLN. INFO.:			EP 2006-123974	A 20061113
			US 2006-60865484	P 20061113
			US 2007-60939140	P 20070521
			EP 2007-822513	A 20071113
			WO 2007-EP62237	W 20071113

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
EP 1921149	A1	Dead	20201201
AU 2007321266	A1	Dead	20221215
AU 2007321266	B2	Dead	20221215
CA 2669219	A1	Dead	20201120
WO 2008058944	A1	Dead	20201201
AR 63795	A1	Alive	20201121
EP 2092067	A1	Indeterminate	20201107
EP 2092067	B1	Indeterminate	20201107
KR 2009092803	A	Alive	20201121
KR 1505413	B1	Alive	20201120
CN 101595219	A	Dead	20230504
CN 101595219	B	Dead	20230504
JP 2010508861	T	Dead	20201120
JP 5479102	B2	Dead	20201120

AT 469971	T	Alive	20201121
PT 2092067	E	Alive	20201120
ES 2347019	T3	Alive	20201121
ME 1846	B	Alive	20201120
US 20110287037	A1	Alive	20230622
US 8669091	B2	Alive	20230622
NZ 576947	A	Alive	20201121
RU 2447145	C2	Alive	20201121
IL 198736	A	Alive	20221222
TW I394834	B	Dead	20201121
BR 2007019003	A2	Dead	20201121
ZA 2009003022	A	Alive	20201121
MX 2009005038	A	Alive	20201121
NO 2009002278	A	Dead	20201121
HK 1138314	A1	Dead	20221215
CY 1111035	T1	Alive	20201120

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB The invention relates to a microorganism as a carrier of nucleotide sequences coding for antigens and protein toxins comprising the following components: (I) at least one nucleotide sequence coding for at least one complete or partial antigen of at least one wild-type or mutated protein; and (II) at least one nucleotide sequence coding for at least one protein toxin and/or at least one protein toxin subunit; and (III) (a) at least one nucleotide sequence coding for at least one transport system which enables the expression of the expression products of component (I) and component (II) on the outer surface of the microorganism and/or enables the secretion of the expression products of component (I) and component (II); and/or (III) (b) optionally, at least one nucleotide sequence coding for at least one protein for lysing the microorganism in the cytosol of mammalian cells and for intracellularly releasing plasmids or expression vectors, which are contained in the lysed microorganism; and (IV) at least one nucleotide sequence for at least one activation sequence for the expression of one or more of components (I) to (III), wherein said activation sequence can be activated in the microorganism and/or is tissue cell-specific, tumor cell-specific, macrophage-specific, dendrite-specific, lymphocyte-specific, function-specific or "non-cell-specific"; wherein any of components (I) to (IV) can be present either once or several times and either identical or different. The invention also discloses a process of manufg. thereof, corresponding plasmids or expression vectors and uses of the microorganism as a medicament. The invention specifically claims use of chimeric proteins cholera toxin subunit B (CtxB)-prostate specific antigen (PSA), CtxB-B-Raf kinase domain, CtxB-B-Raf kinase domain mutants, CtxB-hemagglutinin HA1, and CtxB-hemagglutinin HA12C. The chimeric proteins are expressed under control of an endogenous promoter of the Escherichia coli hly locus and also contain signal sequences from the Hly secretion system. Mice were immunized intragastrically with bacteria expressing a CtxB-PSA chimeric protein and an immune response was obsd. comprising CD8+ T cells and the innate immune system (probably NK cells). The immunized mice showed reduced tumor vol. at 9, 12, and 14 days after tumor challenge by s.c. injection of P815 cell line expressing full-length prostate specific antigen.

OS.CITING REF COUNT: 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
AR 63795 A1	AR 2007-105040	Register
AT 469971 T	AT 2007-822513	Register
AU 2007321266 A1	AU 2007-321266	Register
AU 2007321266 B2	AU 2007-321266	Register
BR 2007019003 A2	BR 2007-19003	Register
CA 2669219 A1	CA 2007-2669219	Register
CN 101595219 A	CN 2007-80044576	Register
CN 101595219 B	CN 2007-80044576	Register
EP 1921149 A1	EP 2006-123974	Register Federated Reg.
EP 2092067 A1	EP 2007-822513	Register Federated Reg.
EP 2092067 B1	EP 2007-822513	Register Federated Reg.
ES 2347019 T3	ES 2007-822513	Register

HK 1138314 A1	HK 2010-100634	Register
IL 198736 A	IL 2007-198736	Register
IN 2009KN01749 A	IN 2009-KN1749	Register
JP 2010508861 T	JP 2009-536716	Register Global Dossier
JP 5479102 B2	JP 2009-536716	Register Global Dossier
KR 1505413 B1	KR 2009-7012089	Register Global Dossier
KR 2009092803 A	KR 2009-7012089	Register Global Dossier
MX 2009005038 A	MX 2009-5038	Register
NO 2009002278 A	NO 2009-2278	Register
NZ 576947 A	NZ 2007-576947	Register
PH 12009500878 B1	PH 2009-500878	Register
PT 2092067 E	PT 2007-822513	Register
RU 2447145 C2	RU 2009-122560	Register
TW I394834 B	TW 2007-142857	Register
US 20110287037 A1	US 2007-939254	Register Global Dossier
US 8669091 B2	US 2007-939254	Register Global Dossier
WO 2008058944 A1	WO 2007-EP62237	Register Global Dossier

L22 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2008:34769 CAPLUS Full-text

DOCUMENT NUMBER: 148:578159

TITLE: Comparative study of FKN in genome and protein expression among human, chimpanzee and leaf monkey

AUTHOR(S): Hong, Xiaowu; Zhang, Yaping; Chu, Yiwei; Gao, Bo; Shao, Xianan; Jiang, Zhenggang; Xiong, Sidong

CORPORATE SOURCE: Department of Immunology, Shanghai Medical College, Fudan University, Shanghai, 200032, Peop. Rep. China

SOURCE: Zhongguo Mianyixue Zazhi (2007), 23(4), 326-330

CODEN: ZMZAEE; ISSN: 1000-484X

PUBLISHER: Zhongguo Mianyixue Zazhi Bianjibu

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB Full-length FKN gene of human, chimpanzee and leaf monkey were cloned and their differences in genome and protein expression level were compared. Three exons of FKN were spliced in turn via gene splicing by overlapping extension PCR and inserted into pcDNA3.1/myc-His(-)A and then transfected into CHO cells via liposome after enzyme digestion and sequencing. The protein expressed was analyzed with RT-PCR, SDS-PAGE and Western blot. Enzyme digestion and sequence anal. showed that the DNA fragments inserted into eukaryotic vectors were correct full-length FKN gene and the target gene could be amplified from CHO cells transfected with recombinant vectors by RT-PCR. Expressed protein in CHO cells could be secreted into supernatant and combine with c-myc antibody specifically. SDS-PAGE showed the appearance of 95000 band of FKN protein. Sequence showed that besides other point mutation, there was an apparent 30 nucleotide deletion mutation in human and chimpanzee FKN when compared to that of leaf monkey. However, the deletion mutation did not affect its protein expression level. In summary, the full-length FKN gene of human, chimpanzee and leaf monkey have been cloned successfully, and the results and their differences in genome and protein expression levels lay the foundation for further study of its evolution in immunol. function in primates.

L22 ANSWER 23 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2006:224714 CAPLUS Full-text

DOCUMENT NUMBER: 145:245961

TITLE: Biological and molecular characterization of a canine hemangiosarcoma-derived cell line

AUTHOR(S): Thamm, Douglas H.; Dickerson, Erin B.; Akhtar, Nasim; Lewis, Rachel; Auerbach, Robert; Helfand, Stuart C.; MacEwen, E. Gregory

CORPORATE SOURCE: Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA

SOURCE: Research in Veterinary Science (2006), 81(1), 76-86

CODEN: RV TSA9; ISSN: 0034-5288

DIGITAL OBJECT ID: 10.1016/j.rvsc.2005.09.005

PUBLISHER: Elsevier B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Canine hemangiosarcoma (HSA) is a devastating disease. Investigation of novel therapies has been limited by the limited availability of canine HSA-derived cell lines. We report the development of a canine HSA-derived cell line, DEN-HSA, which recapitulates features of angiogenic

endothelium. DEN-HSA cells were derived from a spontaneous HSA arising in the kidney of a dog. DEN-HSA displayed surface mols. distinctive of endothelial histogenesis, including factor VIII-related antigen, ICAM-1 and $\alpha v \beta 3$ integrin. In vitro, DEN-HSA formed microvascular tube-like structures on Matrigel, and proliferated in response to a variety of angiogenic growth factors. The cells expressed mRNA and protein specific for bFGF and its receptors, and VEGF and its receptors, among others. DEN-HSA conditioned medium evoked a marked angiogenic response in a murine corneal pocket assay, indicating potent proangiogenic activity of substances secreted by this cell line. This research confirms the DEN-HSA cell line as endothelial in origin, suggests the presence of angiogenic growth factor autocrine loops, and offers the potential to utilize DEN-HSA cells for the study of novel therapies that modulate endothelial proliferation.

OS.CITING REF COUNT: 25 THERE ARE 25 CAPLUS RECORDS THAT CITE THIS RECORD (25 CITINGS)
 REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 24 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2004:32343 CAPLUS Full-text
 DOCUMENT NUMBER: 140:106529
 TITLE: Mouse extracellular matrix proteins, cDNA cloning, and use as cell adhesion agent
 INVENTOR(S): Sekiguchi, Kiyotoshi; Manabe, Riichiro; Hayashizaki, Yoshihide; Kamiya, Mamoru; Furuya, Hiroshi; Tsutsui, Aogu
 PATENT ASSIGNEE(S): Japan Science and Technology Corporation, Japan; Institute of Physical and Chemical Research; DNA Form Co., Ltd.
 ULTIMATE OWNER: DNAFORM KK
 ULTIMATE OWNER STANDARD: DNAFORM KK
 SOURCE: Jpn. Kokai Tokkyo Koho, 86 pp.
 CODEN: JKXXAF
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2004008021	A	20040115	JP 2002-162211	20020603
			JP 2002-162211	20020603

PRIORITY APPLN. INFO.:

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
JP 2004008021	A	Dead	20201121

AB Novel extracellular matrix proteins of mouse origin, encoding cDNAs, recombinant expression, antibodies against them, and use as cell adhesion agent, are disclosed. Genes coding for those extracellular matrix proteins were designated 33145, 40240, 51140, and 31418. Besides secretion signal sequence, 33145 protein had SCP (sperm-coating glycoprotein) domain, LCCL (Limulus factor C, Coch-5b2 and Lgll) domain, and a potential N-glycosylation site. Homol. with Cocoa Crisp (cysteine-rich secreted protein), late gestation lung protein, protease inhibitor 15 preproprotein, 25 kDa trypsin inhibitor and glioma pathogenesis-related protein (RTVP-1 protein) was found. 40420 Protein had 2 potential N-glycosylation sites, and showed homol. with human FLJ23191 gene and mouse BB613765, AV243621, AK017759, and AK020942 genes. 51140 Protein has a thrombospondin type 1 repeats (TSP-1) domain, a potential N-glycosylation site, and a cysteine rich region homologous to that of PACE4 protein (Furin-like protease serine protease), and showed homol. with thrombospondin. 31418 Protein has four tandem repeat sequences, and a sequence homologous to bovine prion protein heparin-binding domain, and showed homol. with monkey Taste Bud-specific protein. The proteins were recombinantly expressed as fusion protein with EGFP-His-myc tag in human 293T cells. Localization in extracellular matrix was detected. Cell adhesion and growth activities were found. Prodn. of anti-40240 antibodies is described.

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER

L22 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2023 ACS on STN

ACCESSION NUMBER: 2000:314830 CAPLUS Full-text

DOCUMENT NUMBER: 132:344143

TITLE: Isolated nucleic acids encoding an *Oreochromis aureus* secretory signal, and vectors composed thereof for expression and secretion of heterologous recombinant proteins

INVENTOR(S): Ding, Jeak Ling; Tan, Nguan Soon; Ho, Bow

PATENT ASSIGNEE(S): National University of Singapore, Singapore; Lam, Toong Jin

SOURCE: PCT Int. Appl., 73 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000026366	A1	20000511	WO 1999-SG108	19991029
US 6733997	B1	20040511	US 1999-426776	19991026
AU 2000010892	A	20000522	AU 2000-10892	19991029
PRIORITY APPLN. INFO.:			US 1998-60106426	P 19981030
			US 1999-426776	A 19991026
			WO 1999-SG108	W 19991029

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2000026366	A1	Dead	20201107
US 6733997	B1	Dead	20201107
AU 2000010892	A	Dead	20201107

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB A universal secretory signal (ss) originally derived from *Oreochromis aureus* vitellogenin (Vtg) gene is inserted into various expression vectors for driving the secretion of a recombinant protein into the culture medium. This enhances the detection, quantification and downstream scaled-up purifn. of a recombinant protein of interest. The secretory signal system is very versatile, being conveniently and widely applicable to an array of heterologous host cells such as bacteria, yeast, insect, piscine, and mammalian cell lines (e.g., COS, CHO, NIH/3T3). The said secretory system is also applicable as a reporter vector for secretion of reporter proteins/enzymes, thus, enabling the detection of the reporter proteins (e.g., CAT, GFP) in the culture medium.

OS.CITING REF COUNT: 6 THERE ARE 6 CAPLUS RECORDS THAT CITE THIS RECORD (6 CITINGS)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

LINK INFORMATION

PUBLICATIONS	APPLICATIONS	REGISTER
AU 2000010892 A	AU 2000-10892	Register
US 6733997 B1	US 1999-426776	Register
WO 2000026366 A1	WO 1999-SG108	Register Global Dossier

L22 ANSWER 26 OF 29 BIOSIS COPYRIGHT (c) 2023 Clarivate Analytics on STN

ACCESSION NUMBER: 2019:546503 BIOSIS Full-text

DOCUMENT NUMBER: PREV201900645710

TITLE: Identification and bioactivity of a granulocyte colony-stimulating factor b homologue from large yellow croaker (*Larimichthys crocea*).

AUTHOR(S): Li, Qihua [Reprint Author]; Xu, Libing; Ao, Jingqun; Ai, Chunxiang [Reprint Author]; Chen, Xinhua [Reprint Author]

CORPORATE SOURCE: Xiamen Univ, Coll Ocean and Earth Sci, Xiamen 361102, Fujian, Peoples R China

SOURCE: chunxai@xmu.edu.cn; chenxinhua@tio.org.cn

Fish & Shellfish Immunology, (JUL 2019) Vol. 90, pp. 20-29.

ISSN: 1050-4648. E-ISSN: 1095-9947.

DIGITAL OBJECT ID: 10.1016/j.fsi.2019.04.048

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 10 Jul 2019
Last Updated on STN: 10 Jul 2019

AB Granulocyte colony-stimulating factor (GCSF) is a pleiotropic cytokine that plays a key role in regulation of hematopoiesis, innate and adaptive immune responses in mammals. However, bioactivity of GCSF in teleost fish remains largely unknown. In this study, a GCSFb homologue from large yellow croaker (*Larimichthys crocea*) (LcGCSFb) was cloned by RACE-PCR techniques. The open reading frame (ORF) of LcGCSFb is 603 bp long and encoded a protein precursor of 200 amino acids (aa), with a 19-aa signal peptide and a 181-aa mature peptide. In healthy fish, the LcGCSFb was constitutively expressed in all examined tissues, with the highest levels in mucous tissues, such as gills, intestine, and stomach. Its transcripts in head kidney, spleen, gills, intestine and stomach were significantly induced by *Vibrio alginolyticus* challenge. LcGCSFb transcripts were also detected in primary head kidney leukocytes (PKL), primary head kidney macrophages (PKM), primary head kidney granulocytes (PKG) and head kidney cell line (LYCK), and markedly upregulated by inactivated *V. alginolyticus*. These data suggested that LcGCSFb may play a role in immune response against bacterial infection. In vivo administration of recombinant LcGCSFb protein (rLcGCSFb) significantly upregulated the expression levels of the inflammatory cytokines (IL-6 and TNF alpha), and transcription factor C/EBP beta, which is required for proliferation of neutrophils. Furthermore, rLcGCSFb showed an ability to strengthen the phagocytosis of PKL in vitro. Taken together, LcGCSFb may be involved in antibacterial immunity via promoting the inflammatory response and the phagocytic activity of leukocytes. To our knowledge, this is the first report on immunoregulatory roles of GCSF in teleost.

L22 ANSWER 27 OF 29 BIOSIS COPYRIGHT (c) 2023 Clarivate Analytics on STN

ACCESSION NUMBER: 2018:120431 BIOSIS Full-text

DOCUMENT NUMBER: PREV201800120431

TITLE: Vascular endothelial growth factor-C ameliorates renal interstitial fibrosis through lymphangiogenesis in mouse unilateral ureteral obstruction.

AUTHOR(S): Hasegawa, Shoko; Nakano, Toshiaki; Torisu, Kumiko; Tsuchimoto, Akihiro; Eriguchi, Masahiro; Haruyama, Naoki; Masutani, Kosuke; Tsuruya, Kazuhiko [Reprint Author]; Kitazono, Takanari

CORPORATE SOURCE: Kyushu Univ, Grad Sch Med Sci, Dept Integrated Therapy Chron Kidney Dis, Higashi Ku, 3-1-1 Maidashi, Fukuoka 8128582, Japan

SOURCE: tsuruya@intmed2.med.kyushu-u.ac.jp
Laboratory Investigation, (DEC 2017) Vol. 97, No. 12, pp. 1439-1452. <http://www.nature.com/labinvest/index.html>.
CODEN: LAINAW. ISSN: 0023-6837. E-ISSN: 1530-0307.

DIGITAL OBJECT ID: 10.1038/labinvest.2017.77

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 17 Jan 2018

Last Updated on STN: 17 Jan 2018

AB Renal fibrosis is the final common pathway of chronic kidney diseases. Lymphatic vessel (LV) proliferation is found in human renal diseases and other fibrotic diseases, suggesting that lymphangiogenesis is associated with the progression or suppression of kidney diseases. However, the purpose of LV proliferation is not completely understood. We investigated the effect of vascular endothelial growth factor (VEGF)-C on lymphangiogenesis, inflammation, and fibrosis in the mouse kidney using the unilateral ureteral obstruction (UUO) model. In UUO mice, significant proliferation of LVs was accompanied by tubulointerstitial nephritis and fibrosis. We continuously administered recombinant human VEGF-C to UUO model mice using an osmotic pump (UUO+ VEGF-C group). Lymphangiogenesis was significantly induced in the UUO+VEGF-C group compared with the vehicle group, despite similar numbers of capillaries in both groups. The number of infiltrating macrophages, and levels of inflammatory cytokines and transforming growth factor-beta 1 were reduced in the UUO+VEGF-C group compared with the vehicle group. Renal fibrosis was consequently attenuated in the UUO+VEGF-C group. In cultured lymphatic endothelial cells, administration of VEGF-C increased the activity and proliferation of lymphatic endothelial cells (LECs) and expression of adhesion molecules such as vascular cell adhesion molecule-1. These findings suggest that induction of lymphangiogenesis ameliorates inflammation and fibrosis in the renal interstitium. Enhancement of the VEGF-C signaling pathway in

LECs may be a therapeutic strategy for renal fibrosis.

L22 ANSWER 28 OF 29 BIOSIS COPYRIGHT (c) 2023 Clarivate Analytics on STN
ACCESSION NUMBER: 2014:22420 BIOSIS Full-text
DOCUMENT NUMBER: PREV201400022420
TITLE: Arginine Deiminase Resistance in Melanoma Cells Is Associated with Metabolic Reprogramming, Glucose Dependence, and Glutamine Addiction.
AUTHOR(S): Long, Yan; Tsai, Wen-Bin; Wangpaichitr, Medhi; Tsukamoto, Takashi; Savaraj, Niramol; Feun, Lynn G.; Kuo, Macus Tien [Reprint Author]
CORPORATE SOURCE: Univ Texas MD Anderson Canc Ctr, 1515 Holcombe Blvd, Box 951, Houston, TX 77030 USA
tkuo@mdanderson.org
SOURCE: Molecular Cancer Therapeutics, (NOV 2013) Vol. 12, No. 11, pp. 2581-2590.
ISSN: 1535-7163. E-ISSN: 1538-8514.
DIGITAL OBJECT ID: 10.1158/1535-7163.MCT-13-0302
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Dec 2013
Last Updated on STN: 11 Dec 2013

AB Many malignant human tumors, including melanomas, are auxotrophic for arginine due to reduced expression of argininosuccinate synthetase-1 (ASS1), the rate-limiting enzyme for arginine biosynthesis. Pegylated arginine deiminase (ADI-PEG20), which degrades extracellular arginine, resulting in arginine deprivation, has shown favorable results in clinical trials for treating arginine-auxotrophic tumors. Drug resistance is the major obstacle for effective ADI-PEG20 usage. To elucidate mechanisms of resistance, we established several ADI-PEG20-resistant (ADI(R)) variants from A2058 and SK-Mel-2 melanoma cells. Compared with the parental lines, these ADI(R) variants showed the following characteristics: (i) all ADI(R) cell lines showed elevated ASS1 expression, resulting from the constitutive binding of the transcription factor c-Myc on the ASS1 promoter, suggesting that elevated ASS1 is the major mechanism of resistance; (ii) the ADI(R) cell lines exhibited enhanced AKT signaling and were preferentially sensitive to PI3K/AKT inhibitors, but reduced mTOR signaling, and were preferentially resistant to mTOR inhibitor; (iii) these variants showed enhanced expression of glucose transporter-1 and lactate dehydrogenase-A, reduced expression of pyruvate dehydrogenase, and elevated sensitivity to the glycolytic inhibitors 2-deoxy-glucose and 3-bromopyruvate, consistent with the enhanced glycolytic pathway (the Warburg effect); (iv) the resistant cells showed higher glutamine dehydrogenase and glutaminase expression and were preferentially vulnerable to glutamine inhibitors. We showed that c-Myc, not elevated ASS1 expression, is involved in upregulation of many of these enzymes because knockdown of c-Myc reduced their expression, whereas overexpressed ASS1 by transfection reduced their expression. This study identified multiple targets for overcoming ADI-PEG resistance in cancer chemotherapy using recombinant arginine-degrading enzymes. Mol Cancer Ther; 12(11); 2581-90. (C) 2013 AACR.

L22 ANSWER 29 OF 29 BIOSIS COPYRIGHT (c) 2023 Clarivate Analytics on STN
ACCESSION NUMBER: 2010:211844 BIOSIS Full-text
DOCUMENT NUMBER: PREV201000211844
TITLE: Cloning and molecular characterization of Dashurin encoded by C20orf116, a PCI-domain containing protein.
AUTHOR(S): Neziri, D.; Ilhan, A.; Maj, M.; Majdic, O.; Baumgartner-Parzer, S.; Cohen, G.; Base, W.; Wagner, L. [Reprint Author]
CORPORATE SOURCE: Med Univ Vienna, Dept Med 3, Waehringer Guertel 18-20, A-1090 Vienna, Austria
ludwig.wagner@meduniwien.ac.at
SOURCE: Biochimica et Biophysica Acta, (APR 2010) Vol. 1800, No. 4, pp. 430-438.
ISSN: 0304-4165. E-ISSN: 1872-8006.
DIGITAL OBJECT ID: 10.1016/j.bbagen.2009.12.004
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: GenBank-NP076424; EMBL-NP076424; DDJB-NP076424
ENTRY DATE: Entered STN: 21 Apr 2010
Last Updated on STN: 21 Apr 2010

AB Background: Characterization of gene products originating from undefined open reading frames and delineation of biological functions has become the task after the human genome has been decoded. Methods: We cloned the human C20orf 116 and defined its transcript in liver, kidney and various brain regions by Northern analysis. Antibodies against recombinant protein used for immunofluorescence and immunoblots confirmed its expression in these tissues. With the focus on kidney, its tubular expression and presence in glomerula were shown. Results: A 28 aa long signal peptide predicted by in silico analysis is reflected by visualization of size variants of similar to 3 kDa difference suggesting a signal peptidase cleavage of the proform. Cell compartment separation confirmed the presence of Dashurin in peroxisomes/mitochondria, microsomes, cytosol and nucleus. This is in line with green fluorescent protein (GFP)-Dashurin fusion protein shuttling between cytosol and nucleus. Luciferase reporter studies revealed a 2-3 fold increase of promoter activities upon over-expression. Bioinformatic analysis identified a PCI-domain at the C-terminus providing protein-protein interaction capabilities. Conclusion: Our present findings suggest the involvement of Dashurin in gene transcription or mRNA translation. General significance: Dashurin shares the PCI-domain with three multisubunit protein complexes (26S proteasome, COP9 signalosome and eIF3 translation initiation factor). (c) 2009 Elsevier B.V. All rights reserved.

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(FILE 'HOME' ENTERED AT 05:42:13 ON 12 SEP 2023)
D CLUSTER
D CLUSTER .DJS
D SET

FILE 'CAPLUS' ENTERED AT 05:42:36 ON 12 SEP 2023
E MIZUMURA HIKARU/AU 25
L1 26 SEA "MIZUMURA HIKARU"/AU
E ODA TOSHIO/AU 25
L2 94 SEA "ODA TOSHIO"/AU
E KAWABATA SHUN/AU 25
L3 144 SEA "KAWABATA SHUN ICHIRO"/AU
L4 247 SEA L1 OR L2 OR L3
L5 3193236 SEA FACTOR
L6 72 SEA L4 AND L5
L7 949971 SEA (SECRET? OR EXCRET?)
L8 8 SEA L6 AND L7
D IBIB ABS 1-
L9 9 SEA L6 AND RECOMBINANT
L10 9 SEA L9 NOT L8
D IBIB ABS 1-
L11 3 SEA L6 AND (CHO OR HEK?)
L12 0 SEA L6 AND (CHINESE OR KIDNEY)
D IBIB ABS 1- L11

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT
05:51:39 ON 12 SEP 2023
L13 95203 SEA "FACTOR C"
L14 671016 SEA (CHO OR HEK?)
L15 6689580 SEA (CHINESE OR KIDNEY)
L16 7108972 SEA L14 OR L15
L17 5621 SEA L13 AND L16
L18 258 SEA L17 AND RECOMBINANT?
L19 5389518 SEA (SECRET? OR EXCRET?)
L20 37 SEA L18 AND L19
L21 29 DUP REM L20 (8 DUPLICATES REMOVED)
L*** DEL 6 S L18 AND L19
L*** DEL 6 S L18 AND L19
L*** DEL 22 S L18 AND L19
L*** DEL 6 S L18 AND L19
L*** DEL 6 S L18 AND L19
L*** DEL 6 S L18 AND L19
L22 29 SEA L21 NOT (L8 OR L9)
D IBIB ABS 1-

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FILE CAPLUS

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FILE COVERS 1907 - 12 Sep 2023 VOL 184 ISS 1

FILE LAST UPDATED: 11 Sep 2023 (20230911/ED)

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This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE MEDLINE

FILE COVERS 1946 TO DATE.

FILE LAST UPDATED: 11 Sep 2023 (20230911/UP).

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Effective December 29, 2022, the 2023 version of the MeSH thesaurus is now available in Medline. The MeSH thesaurus is a great resource for the latest biomedical search terminology.

This file contains CAS Registry Numbers for easy and accurate substance identification. See HELP CASRN within Medline for further information.

See HELP RANGE before carrying out any RANGE search.

FILE AGRICOLA

FILE LAST UPDATED: 10 Aug 2023 (20230810/UP)

FILE COVERS 1970 TO DATE

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION IS AVAILABLE IN THE BASIC INDEX (/BI), ABSTRACT (/AB), AND TITLE (/TI) FIELDS <<<

FILE BIOSIS

FILE COVERS 1926 TO DATE.

CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNS) PRESENT FROM JANUARY 1926 TO DATE.

RECORDS LAST ADDED: 6 September 2023 (20230906/ED)

BIOSIS has been augmented with 1.8 million archival records from 1926 through 1968. These records have been re-indexed to match current BIOSIS indexing.

FILE EMBASE

FILE COVERS: Embase-originated material 1947 to 11 Sep 2023 (20230911/ED)
Unique MEDLINE content 1948 to present

Information on the current version of Emtree, the EMBASE thesaurus,
is available in HELP EMTREE.

This file contains CAS Registry Numbers for easy and accurate
substance identification.

FILE SCISEARCH

FILE COVERS 1974 TO 11 Sep 2023 (20230911/ED)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/346,420	07/03/2023	Hikaru MIZUMURA	Q289014	6959
23373	7590	09/01/2023	EXAMINER	
SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 9000 WASHINGTON, DC 20006			CENTRAL, DOCKET	
			ART UNIT	PAPER NUMBER
			OPAP	
			NOTIFICATION DATE	DELIVERY MODE
			09/01/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PPROCESSING@SUGHRUE.COM
USPTO@sughrue.com
sughrue@sughrue.com

<i>Decision Granting Request for Prioritized Examination (Track I)</i>	Application No. 18/346,420	Applicant(s) MIZUMURA et al.	
	Examiner FIKIRTE A GEREMEW	Art Unit OMBL	AIA (FITF) Status Yes
<p>1. THE REQUEST FILED <u>03 July 2023</u> IS GRANTED .</p> <p>The above-identified application has met the requirements for prioritized examination</p> <p>A. ☒ for an original nonprovisional application (Track I).</p> <p>B. ☐ for an application undergoing continued examination (RCE).</p> <p>2. The above-identified application will undergo prioritized examination. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:</p> <p>A. filing a <u>petition for extension of time</u> to extend the time period for filing a reply;</p> <p>B. filing an <u>amendment to amend the application to contain more than four independent claims, more than thirty total claims</u>, or a multiple dependent claim;</p> <p>C. filing a <u>request for continued examination</u> ;</p> <p>D. filing a notice of appeal;</p> <p>E. filing a request for suspension of action;</p> <p>F. mailing of a notice of allowance;</p> <p>G. mailing of a final Office action;</p> <p>H. completion of examination as defined in 37 CFR 41.102; or</p> <p>I. abandonment of the application.</p> <p>Telephone inquiries with regard to this decision should be directed to FIKIRTE GEREMEW at (703) 756-1930. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.</p>			
/FIKIRTE A GEREMEW/ PROGRAM SUPPORT ASSISTANT, OMBL			

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q289014

Hikaru MIZUMURA, et al.

Appln. No.: 18/346,420

Group Art Unit: OPAP

Confirmation No.: 6959

Examiner: CENTRAL, DOCKET

Filed: July 3, 2023

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

**INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§ 1.97 and 1.98**

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure under 37 C.F.R. § 1.56, Applicant hereby
notifies the U.S. Patent and Trademark Office of the documents which are listed on the attached
PTO/SB/08.

One copy of each of the listed documents is submitted herewith, except for the following:
U.S. patents and/or U.S. patent publications.

The present Information Disclosure Statement is being filed: (1) No later than three
months from the application's filing date; (2) Before the mailing date of the first Office Action
on the merits (whichever is later); or (3) Before the mailing date of the first Office Action after
filing a request for continued examination (RCE) under §1.114, and therefore, no Statement
under 37 C.F.R. § 1.97(e) or fee under 37 C.F.R. § 1.17(p) is required.

INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§ 1.97 and 1.98
Appln. No.: 18/346,420

Attorney Docket No.: Q289014

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/markboland/

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: August 18, 2023

Mark Boland
Registration No. 32,197

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	OPAP
	Examiner Name	CENTRAL, DOCKET
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	1.	6733997	B1	2004-05-11	Ding et al.	

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	2.	Extended European Search Report issued July 4, 2023 in European Application No. 22215393.4	English
	3.	Decision dated October 26, 2022 issued in European Application No. 18190686.8	English
	4.	Neus FERRER-MIRALLES et al., "General Introduction: Recombinant Protein Production and Purification of Insoluble Proteins", Methods and Protocols, Methods in Molecular Biology, 2015, Vol. 1258, pp. 1-24 (24 pages total)	English
	5.	Opponent's Reply to the Grounds of Appeal filed by the Proprietor dated July 12, 2023 filed in corresponding European Patent No. 3 441 466	English

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 1 of 2]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	18/346,420
Confirmation Number	6959
Filing Date	July 3, 2023
First Named Inventor	Hikaru MIZUMURA
Art Unit	OPAP
Examiner Name	CENTRAL, DOCKET
Attorney Docket Number	Q289014

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

- ☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

- ☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).
- ☐ See attached certification statement.
- ☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
- ☒ The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.
- ☒ A certification statement is not submitted herewith.

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/markboland/	Date (YYYY-MM-DD)	2023-08-18
Name/Print	Mark Boland	Registration Number	32,197

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 2 of 2]



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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
08/18/2023 05:47:31 PM ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY J oy Martinez

PATENT CENTER # 62642116

FILING DATE 07/03/2023

CUSTOMER # 23373

FIRST NAMED INVENTOR Hikaru MIZUMURA

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Hui Wauters

Documents

TOTAL DOCUMENTS: 7

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Q289014IDSasFiled.pdf	4	-	172 KB
Q289014IDSasFiled-TRAN.LET.pdf (1-2)	2	Transmittal Letter	87 KB
Q289014IDSasFiled-IDS.pdf (3-4)	2	Information Disclosure Statement (IDS) Form (SB08)	132 KB
Q289014SupplementalIDSasFiled.pdf	4	Information Disclosure Statement (IDS) Form (SB08)	162 KB

Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.

Ref 2 EESR dated J uly 4 2023 issued in EP22215393.4.pdf	10	Non Patent Literature	697 KB
Ref 3 Decision dated October 26 2022 revoking corresponding EP Patent No. 3441466.pdf	38	Non Patent Literature	2135 KB
Ref 4 Insoluble Proteins Methods and Protocols Methods in Molecular BiologyVol. 1258 pp 1-242015.pdf	24	Non Patent Literature	2245 KB
Ref 5 Opponent_s Reply dated J uly 12 2023 filed in corresponding EP Patent No. 3441466.pdf	104	Non Patent Literature	1722 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Q289014IDSasFiled.pdf	30331ED447345D0A6CFEE7ADF8D2927D05233A633A28788358 279FB192AB820682EE7B791F1A69FECDD7CC29707D194B7492 60B5DE26FC482E2B5138484CA17ED
Q289014IDSasFiled- TRAN.LET.pdf	7BA4768E0E88F676FC577C0A99C7FB2BBC7953EF20254C0A2 746612AA051A9350AF79C54C6120C63A12ACBEBE56A60615F 888934BC798C2BF564CCA441DE79EA
Q289014IDSasFiled-IDS .pdf	DC9D4B7920A37F07490750FDB84EC3EFE9FF5EA293671EFBA 4794D51B8A441116CAB84C112FE904635C80A00AE32C2E6D9 A5B03FE2EDF3349AB33F53490B2AEE
Q289014SupplementalIDSasFil ed.pdf	71FF23333367FFC738992C9B132104D3A754AD05E8FFEDD46 BA4FBBE83ACB66E35F587F78190532925F5887A97A1A61022C 66DD787182818F6181573CC0726F2

Ref 2 EESR dated J uly 4 2023 issued in EP22215393.4.pdf	DC9026578F1F9EA010F14B17F973771D9819E98DB3E2BB270 D3D48801A55B76F01CC2C9BF82CEB5076733FE210F59C5B2F 18B4640375A31D69D599B2F261A534
Ref 3 Decision dated October 26 2022 revoking corresponding EP Patent No. 3441466.pdf	D930BAB237D9E77F43EC440D9BE98AB1717339E4FDBF381F4 6DA79A6BAE0656BCEB87B07FDDDFAFDF86A330B6F6B02DC 0118D76139A8D477C6E6BE2170981155
Ref 4 Insoluble Proteins Methods and Protocols Methods in Molecular BiologyVol. 1258 pp 1-242015.pdf	BB8F89643894F95FC24489676CCCFA7EA4C6C151476747C8F EF785B18D16F11D87DF29B4E53319DA332585869E8952EBE47 C7B088418BDD15BA4FF8B6F1F9EAA
Ref 5 Opponent_s Reply dated J uly 12 2023 filed in corresponding EP Patent No. 3441466.pdf	4C94AFEE7574EBB706F20DFED8009167EDC312F86D01E5569 9411060A998632B2DE8A29837414705EA4B1031BB6F5F0B2A5 A4807ACA53CDA0A96B34B3EA24BEA

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q289014

Hikaru MIZUMURA, et al.

Appln. No.: 18/346,420

Confirmation No.: 6959

Examiner: CENTRAL, DOCKET

Filed: July 3, 2023

Group Art Unit: OPAP

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

SUBMISSION OF CORRECTED FORM PTO/SB/08

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Supplemental to the Information Disclosure Statement filed on July 3, 2023, Applicant submits herewith a corrected Form PTO/SB/08 to correct the following inadvertent errors.

Cite No. 20

Indication of “Corresponds to cite 19; Cited in cite 89” in column “T” is corrected to be “Corresponds to cite 11; Cited in cite 84 (ISR)”.

Cite No. 23

Indication of “Corresponds to cite 1 and 7; Cited in cite 89” in column “T” is corrected to be “Corresponds to cite 1 and 4; Cited in cite 84 (ISR)”.

Cite No. 24

Indication of “Cited in cite 89” in column “T” is corrected to be “Cited in cite 84 (ISR)”.

Cite No. 27

Indication of “Corresponds to cite 3; Cited in cite 89” in column “T” is corrected to be “Corresponds to cite 13; Cited in cite 84 (ISR)”.

Cite No. 29

Indication of “Cited in cite 89” in column “T” is corrected to be “Cited in cite 84 (ISR)”.

Cite No. 80

Indication of "Cited in cite 89" in column "T" is corrected to be "Cited in cite 84 (ISR)".

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/markboland/

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: August 18, 2023

Mark Boland
Registration No. 32,197

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	OPAP
	Examiner Name	CENTRAL, DOCKET
	Attorney Docket Number	Q289014

U.S. PATENTS						
Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵
	20.	2012/118226	WO	A1	2012-09-07	SEIKAGAKU CORPORATION		Corresponds to cite 11 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	23.	2006-87435	JP	A	2006-04-06	SEIKAGAKU KOGYO CO LTD		English Abstract; Corresponds to cites 1 and 4 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	24.	94673	SG	A1	2003-03-18	NATIONAL UNIVERSITY OF SINGAPORE		Cited in cite 84 (ISR) in the July 3, 2023 IDS
	27.	2008/004674	WO	A1	2008-01-10	SEIKAGAKU CORPORATION		English Abstract; Corresponds to cite 13 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS
	29.	2005-500520	JP	A	2005-01-06	CAMBREX BIO SCIENCE WALKERVILLE INC		Corresponds to cites 14 and 18 in the July 3, 2023 IDS; Cited in cite 84 (ISR) in the July 3, 2023 IDS

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 1 of 2]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	18/346,420
	Confirmation Number	6959
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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	OPAP
	Examiner Name	CENTRAL, DOCKET
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	80.	XIANGJUN et al., "Role of domains in factor C from Tachypleus tridentatus in neutralization endotoxin", "Accession:2006:239467" File CAPLUS, [online], Retrieved from the STN on the Web, [retrieved on 2014-01-29]; Shengwu Huaxue Yu Shengwu Wuli Jinzhan, Vol. 31, No. 8, pp. 736-740, 2004	Cited in cite 84 (ISR) in the July 3, 2023 IDS

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 2 of 2]



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO.	TOT CLAIMS	IND CLAIMS
18/346,420	07/03/2023		1820	Q289014	10	1

CONFIRMATION NO. 6959

FILING RECEIPT



0000000059877322

23373
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC 20006

Date Mailed: 07/31/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Hikaru MIZUMURA, Tokyo, JAPAN;
Toshio Oda, Tokyo, JAPAN;
Shun-Ichiro Kawabata, Fukuoka, JAPAN;

Applicant(s)

Seikagaku Corporation, Tokyo, JAPAN;

Assignment For Published Patent Application

Seikagaku Corporation, Tokyo, JAPAN

Power of Attorney: The patent practitioners associated with Customer Number 23373

Domestic Priority data as claimed by applicant

This application is a CON of 17/554,128 12/17/2021
which is a CON of 16/399,386 04/30/2019 PAT 11,236,318
which is a CON of 15/983,725 05/18/2018 PAT 10,982,202
which is a CON of 14/650,767 06/09/2015 PAT 10,144,923
which is a 371 of PCT/JP2013/083082 12/10/2013

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.)
JAPAN 2012-269840 12/10/2012 No Access Code Provided

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/27/2023

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/346,420**

Projected Publication Date: 11/09/2023

Non-Publication Request: No

Early Publication Request: No

Title

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND
METHOD FOR MEASURING ENDOTOXIN

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

page 2 of 4

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD						Application or Docket Number 18/346,420					
Substitute for Form PTO-875											
APPLICATION AS FILED - PART I											
		(Column 1)	(Column 2)		SMALL ENTITY		OR	OTHER THAN SMALL ENTITY			
FOR		NUMBER FILED		NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)		
BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A		N/A	N/A			N/A	320		
SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A		N/A	N/A			N/A	700		
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A		N/A	N/A			N/A	800		
TOTAL CLAIMS (37 CFR 1.16(i))		10	minus 20 =	*			OR	x	= 0		
INDEPENDENT CLAIMS (37 CFR 1.16(h))		1	minus 3 =	*				x	= 0		
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).								0		
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))									0		
					TOTAL			TOTAL	1820		
* If the difference in column 1 is less than zero, enter "0" in column 2.											
APPLICATION AS AMENDED - PART II											
		(Column 1)	(Column 2)		(Column 3)		SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=	OR	x	=	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=	OR	x	=	
	Application Size Fee (37 CFR 1.16(s))							OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR			
					TOTAL ADD'L FEE			OR	TOTAL ADD'L FEE		
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AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
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	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=	OR	x	=	
	Application Size Fee (37 CFR 1.16(s))							OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR			
					TOTAL ADD'L FEE			OR	TOTAL ADD'L FEE		
<p>* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.</p> <p>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".</p> <p>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".</p> <p>The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.</p>											

Application Number: 18346420

Reviewed by: 97ClN/eHe4jhmn5GMimwpAP70alPRU2kNiDwvOWHBrv8bqgC4kdzMg==

Note: This validation report concerns the sequence listing filed 07-03-2023 13:25:41 and indicates that NO errors were found by the USPTO during validation of the sequence listing. This validation report should not be construed as meaning that the sequence listing complies with all of the requirements of 37 CFR §§ 1.821-1.825, 37 CFR §§ 1.831-1.835 and 1.839, WIPO Standard ST.25, or WIPO Standard ST.26.

For any sequence listing related questions or concerns, the Applicant is invited to contact the USPTO Sequence Listing Help Desk at 571-272-2510 or SequenceHelpDesk@USPTO.GOV.

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Validated By CRFValidator v 1.5.0

Application No: 18346420

Version No: 1.1

CRF Copy Request from application

Version :

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No. of SeqIDs Defined: 10

Actual SeqID Count: 10

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q289014

Hikaru MIZUMURA, et al.

Appln. No.: Continuation of U.S. Application No. 17/554,128

Confirmation No.: Not Yet Assigned

Examiner: DAVID J STEADMAN (Parent)

Filed: July 3, 2023

Group Art Unit: 1656 (Parent)

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

**INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §§ 1.97 and 1.98**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure under 37 CFR §1.56, Applicant hereby notifies the U.S. Patent and Trademark Office of the documents which are listed on the attached PTO/SB/08 A & B (modified) form which are all the references of record in parent application No. 17/554,128. Applicant is not submitting duplicate copies of these references but requests that they be listed on the face of any patent granted on the above application. (See 37 CFR §1.98(d)).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Respectfully submitted,

/Hui C. Wauters/

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WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: July 3, 2023

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	Not Yet Assigned
Confirmation Number	Not Yet Assigned
Filing Date	July 3, 2023
First Named Inventor	Hikaru MIZUMURA
Art Unit	1656 (Parent)
Examiner Name	DAVID J STEADMAN (Parent)
Attorney Docket Number	Q289014

U.S. PATENTS

Examiner Initials*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	1.	6077946	A	2000-01-20	Iwanaga et al.	
	2.	10144923	B2	2018-12-04	Mizumura et al.	
	3.	5716834	A	1998-02-10	Ding et al.	
	4.	5795962	A	1998-08-18	Iwanaga et al.	
	5.	5840510	A	1998-11-24	Tanaka et al.	
	6.	6645724	B1	2003-11-11	Ding et al.	
	7.	5858706	A	1999-01-12	Ding et al.	
	8.	5712144	A	1998-01-27	Ding et al.	
	9.	10982202	B2	2021-04-20	Mizumura et al.	
	10.	11236318	B2	2022-02-01	Mizumura et al.	

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initials*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear
	11.	20140024062	A1	2014-01-23	Mizumura et al.	
	12.	20130295613	A1	2013-11-07	Kishishita et al.	
	13.	20090208995	A1	2009-08-20	Tamura et al.	
	14.	20040235080	A1	2004-11-25	Chen et al.	
	15.	20020068325	A1	2002-06-06	Ng et al.	
	16.	20100086967	A1	2010-04-08	Kaufmann et al.	
	17.	20140249082	A1	2014-09-04	Cottingham et al.	
	18.	20030054432	A1	2003-03-20	Chen et al.	
	19.	20080131929	A1	2008-06-05	Prentice et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, where Relevant Passages or Relevant Figures Appear	T ⁵
	20.	2012/118226	WO	A1	2012-09-07	SEIKAGAKU CORPORATION		Corresponds to cite 19; Cited in cite 89
	21.	2008-520250	JP	A	2008-06-19	BIOGEN IDEC MA INC.,		Cited in JPOA 8/7/18 in no. 2014-552048; Corresponds to US 2008/0131929 A1

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

[Page 1 of 6]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	Not Yet Assigned
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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

FOREIGN PATENT DOCUMENTS								
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	22.	9915676	WO	A1	1999-04-01	National University of Singapore		
	23.	2006-87435	JP	A	2006-04-06	SEIKAGAKU KOGYO CO LTD		English Abstract; Corresponds to cites 1 and 7; Cited in cite 89
	24.	94673	SG	A1	2003-03-18	NATIONAL UNIVERSITY OF SINGAPORE		Cited in cite 89
	25.	2012091124	WO	A1	2012-07-05	CHUGAI SEIYAKU KABUSHIKI KAISHA		Abstract; Corresponds to US 20130295613; Cited in JP comm. dated 8/7/18 in 2014-552048
	26.	2008520250	JP	A	2008-06-19	BIOGEN IDEC MA INC.		Corresponds to US 20080131929; Cited in JP comm. dated 8/7/18 in 2014-552048
	27.	2008/004674	WO	A1	2008-01-10	SEIKAGAKU CORPORATION		English Abstract; Corresponds to cite 3; Cited in cite 89
	28.	2010-516264	JP	A	2010-05-20	BOEHRINGER INGELHEIM PHARMA GMBH & CO. KG,		Cited in JPOA 8/7/18 in no. 2014-552048; Corresponds to US 2010/0086967 A1
	29.	2005-500520	JP	A	2005-01-06	CAMBREX BIO SCIENCE WALKERVILLE INC		Corresponds to cites 14 and 18; Cited in cite 89

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	30.	A certified translation of the priority document JP 2012269840 filed December 10, 2012	
	31.	Excerpt from Essential Cell Biology (2nd Edition), Alberts et al., Taylor and Francis Group, New York and London, 2004 (page 209)	
	32.	Harada-Suzuki, T. et al., "Further Studies on the Chromogenic Substrate Assay Method for Bacterial Endotoxins Using Horseshoe Crab (Tachypleus tridentatus) Hemocyte Lysate", J. Biochem., 1982, 92(3): 793-800	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	33.	Jeak Ling Ding et al., "Chapter 9, Endotoxin Detection - from Limulus Amebocyte Lysate to Recombinant Factor C ", Endotoxins: Structure, Function and Recognition SERIES : SUBCELLULAR BIOCHEMISTRY IN: SUBCELLULAR BIOCHEMISTRY: REVIEWS AND ESSAYS DEALING WITH THE FUNCTION, GENETICS, BIOGENESIS AND EVOLUTION OF SUB-CELLULAR COMPONENTS, vol. 53, January 1, 2010, XP009510157, 22 pages total, pp. 187-208	
	34.	Nakamura T. et al. "Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in Limulus hemocytes" European Journal of Biochemistry vol. 154, 1986; pages 511-521	
	35.	Office Action dated February 12, 2020 issued in US Patent Application No. 15/983,725	
	36.	The Wikipedia site relating to "Horseshoe Crab" as of Nov. 23, 2011, provided by the WayBack Machine	
	37.	Declaration by Dr. Hikaru Mizumura submitted to the USPTO, signed Feb. 22, 2018 in US14/650767	
	38.	Slade et al., "Identifying the CHO Secretome using Mucin-type O-Linked Glycosylation and Click-chemistry", J. Prot. Res., 11:6175-6186, 2012	
	39.	Bram Blomme et al., "Alteration of protein glycosylation in liver diseases", Journal of Hepatology, 2009, Vol. 50, pages 592-603 (12 pages total)	
	40.	Extended European Search Report dated January 10, 2019 issued by the European Patent Office in application no. 18190686.8	
	41.	Hossler, P. et al., "Optimal and consistent protein glycosylation in mammalian cell culture", Gycobiol. 19(9): 936-949, 2009	
	42.	JING WANG et al., "Functional expression of full length Limulus Factor C in stably transformed Sf9 cells", Biotechnology Letters, 2001, pp. 71-76, Vol. 23	
	43.	Nettleship, Joanne E., "Structural Biology of Glycoproteins", Glycosylation, 2012, p. 41-62. Available from: https://www.intechopen.com/books/glycosylation/structural-biologyof-glycoproteins ;	
	44.	Stephanie Holst et al., "Profiling of different pancreatic cancer cells used as models for metastatic behaviour shows large variation in their N-glycosylation" Scientific Reports, November 30, 2017, Vol. 7, No. 16623, Pages 1-15 (15 pages total)	
	45.	Tomiya, N. et al., "Comparing N-glycan processing in mammalian cell lines to native and engineered lepidopteran insect cell lines", Glycoconjugate Journal, 2004, 21:343-360	
	46.	Office Action dated April 27, 2020 issued in US Patent Application No. 16/399,575	
	47.	Mizumura, et al., Genetic engineering approach to develop next-generation reagents for endotoxin quantification; Innate Immunity, vol. 23, no. 2, pp. 136-146, 2017	
	48.	Damasceno et al., "Protein secretion in Pichia pastoris and advances in protein production", Appl Microbiol Biotechnol (2012) 93: 31-39, Published online: November 2011	
	49.	Office Action issued in the corresponding European Application No. 13862466.3 dated November 30, 2017	
	50.	Tokunaga F. et al. "Lipopolysaccharide-sensitive serine-protease zymogen factor C of horseshoe crab hemocytes identification and alignment of proteolytic fragments produced during the activation show that it is a novel type of serine protease" European Journal of Biochemistry vol. 167 no. 3, 1987; pages 405-416	
	51.	Sequence alignment results for Tachypleus tridentatus vs. Limulus polyphemus, cited in Submission from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	52.	Roche, "DIG Glycan Differentiation Kit Instructions", 2018, Cat. No. 11 210 238 001, pp. 1-5 (5 pages total)	
	53.	Snapshot of the Wikipedia article for "Intravenous sodium bicarbonate (sodium hydrogencarbonate)", as of April 25, 2013 (3 pages total)	
	54.	Su Chen et al., "Production of Recombinant Proteins in Mammalian Cells", Current Protocols in Protein Science, 1998, pp. 5.10.1-5.10.41 (41 pages total)	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3).

³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

<p align="center">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</p> <p align="center">(Not for submission under 37 CFR 1.99)</p>	Application Number	Not Yet Assigned
	Confirmation Number	Not Yet Assigned
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	55.	"SignalP results for Carcinoscopus rotundicauda", cited in Submission from the Opponent dated September 1, 2022 in European Patent No. 3 441 466	
	56.	"SignalP results for Tachypleus tridentatus", cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	57.	Takao Hashiguchi et al., "Expression of Recombinant Proteins in Cultured Human Cells Standard Protocol with 293 strain cells", Protein Science Society Archive, 2008, 1, e017, pp. 1-9 (18 pages total)	Machine Translation; cited in Submission from the opponent dated 9/1/22 in EP Patent No. 3 441 466
	58.	"FAQ-Why do I see too many bands on my Western blot", available from: https://sysy.com/resources/faq/too-many-bands-on-a-western-blot , Synaptic Systems, cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	59.	Communication dated August 10, 2022 regarding the submission filed by the opponent in the EP Patent No. 3441466 on August 4, 2022	
	60.	Merriam-Webster entry for the term "intracellular", cited in Submissions from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	61.	Ina Kraus, "Das Matrixprotein des Virus der Bornaschen Krankheit (The matrix protein of the virus of Borna disease)", 2002 (132 pages total)	Partial Translation; cited in Submission of the opponent dated 8/4/22 in EP Patent No. 3441466
	62.	"Overview of Cell Lysis and Protein Extraction", ThermoFisher Scientific, available from https://www.thermofisher.com/de/de/home/life-science/protein-biology/protein-biologylearning-center/protein-biology-resource-library/pierce-protein-methods/overview-celllysis-and-protein-extraction.html , cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466 (7 pages total)	
	63.	Khar Heng Choo et al., "A Comprehensive assessment of N-terminal signal peptides prediction methods", BMC Bioinformatics, 2009, 10(Suppl 15):S2, pp.1-12 (12 pages total)	
	64.	NCBI Database entry for the Factor C sequence derived from Carcinoscopus rotundicauda, NCBI, retrieved August 23, 2022 from https://www.ncbi.nlm.nih.gov/protein/AAB34361.1 , GenBank: AAB34361.1 (2 pages total)	
	65.	Invitrogen, "FreeStyle 293 Expression System", User Manual, 2010, 23 pages total	
	66.	"Troubleshooting Tips-WB", ABCAM, 2011 (3 pages total)	
	67.	Submission filed by the opponent in the EP Patent No. 3441466 dated September 1, 2022	
	68.	Decision of the Technical Board of Appeal dated May 3, 2000 issued in European Application No. 83301269.3	
	69.	Submission dated April 5, 2018 in the European Application No. 13 862 466.3	
	70.	David Gray, "Overview of Protein Expression by Mammalian Cells", Current Protocols in Protein Science, 1997, pp. 5.9.1-5.9.18 (18 pages total)	
	71.	Henrik Nielsen et al., "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites", Protein Engineering, 1997, Vol. 10, No.1, pp.1-6 (6 pages total)	

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	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	72.	Jeak L. Ding et al., "Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, <i>Carcinoscorpius rotundicauda</i> ", Molecular Marine Biology and Biotechnology, 1995, Vol. 4, No. 1, pp. 90-103 (14 pages total)	
	73.	Invitrogen, "FreeStyle Max Reagent", User Guide, 2013, pp.1-3 (3 pages total)	
	74.	Claire Moore, "Introduction to Western Blotting", 2009, pp. 1-48 (26 pages total)	
	75.	Shengwu Wuli Jinzhan Vol.31, No.8, p736-740, 2004	
	76.	Communication dated April 19, 2017, from the State Intellectual Property Office of the P.R.C., in Chinese application no. 201380064649.3	Translation
	77.	Final Office Action dated August 4, 2020 issued in U.S. Application 16/399,575	
	78.	JEAK L. DING et al., "A new era in pyrogen testing", TRENDS in Biotechnology, August 2001, pp. 277-281, Vol. 19, No. 8	
	79.	Knappskog et al., "The level of synthesis and secretion of <i>Ganssia princeps</i> luciferase in transfected CHO cells is heavily dependent on the choice of signal peptide", Journal of Biotechnology, Vol. 128, 2007, 705-715	
	80.	XIANGJUN et al., "Role of domains in factor C from <i>Tachypleus tridentatus</i> in neutralization endotoxin", "Accession:2006:239467" File CAPLUS, [online], Retrieved from the STN on the Web, [retrieved on 2014-01-29]; Shengwu Huaxue Yu Shengwu Wuli Jinzhan, Vol. 31, No. 8, pp. 736-740, 2004	Cited in cite 89
	81.	The Wikipedia site relating to "Blood Plasma" as of Nov. 19, 2012, provided by the WayBack Machine	
	82.	Declaration by Dr. Hikaru Mizumura submitted to the USPTO, signed Oct. 11, 2017 in US14/650767	
	83.	Koshiba et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria", J. Biol. Chem., 282:3962-3967, 2007	
	84.	International Search Report for PCT/JP2013/083082 dated February 10, 2014	English Translation
	85.	Communication dated July 5, 2016, from the European Patent Office in European Application No. 13862466.3	
	86.	TAKANORI NAKAMURA et al., "Purification and Properties of Intracellular Clotting Factor, Factor B, from Horseshoe Crab (<i>Tachypleus tridentatus</i>) Hemocytes", J. Biochem, 1986, Vol. 99, No. 3, pp. 847-857	
	87.	International Preliminary Report on Patentability in International Application No. PCT/JP2013/083082 issued June 16, 2015 and mailed June 25, 2015	
	88.	Carlesso, E. et al., "The rule regulating pH changes during crystalloid infusion", Intensive Care Med., 2011, 37(3): 461-468	
	89.	Final Office Action dated August 28, 2020 issued in U.S. Application 15/983,725	
	90.	Inhibition data performed using a Limulus Factor C expressed in HEK 293 cells and HEK 293 GnTI- cells filed with Opposition against EP3441466B dated November 5, 2020	
	91.	JING WANG et al., "Modular Arrangement and Secretion of a Multidomain Serine Protease", The Journal of Biological Chemistry, September 27, 2002, pp. 36363-36372, Vol. 277, No. 39	
	92.	Office Action dated May 21, 2020 issued in U.S. Application No. 15/983,725	
	93.	Communication of Notice of Opposition dated November 11, 2020, issued by the European Patent Office in EP Application no. 18190686.8	
	94.	Thomas, P. and Smart, T.G., "HEK293 cell line: A vehicle for the expression of recombinant proteins", J. Pharmacol. And Toxicol. Methods, 2005, 51: 187-200	
	95.	Strategies for Protein Purification Handbook, GE Healthcare, September 2010, 167 pages	
	96.	Written Opinion for PCT/JP2013/083082 dated February 10, 2014	

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[Page 5 of 6]

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	Confirmation Number	Not Yet Assigned
	Filing Date	July 3, 2023
	First Named Inventor	Hikaru MIZUMURA
	Art Unit	1656 (Parent)
	Examiner Name	DAVID J STEADMAN (Parent)
	Attorney Docket Number	Q289014

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	97.	Communication dated July 29, 2016 from the State Intellectual Property Office of the P.R.C. in counterpart application no. 201380064649.3	
	98.	SADAAKI IWANAGA, "The limulus clotting reaction", Current Opinion in Immunology 1993, pp. 74-82, Vol. 5	
	99.	Current Protocols in Molecular Biology (1993) 16.23.1-16.23.13, John Wiley and Sons, Inc., 2002	
	100.	Communication dated August 7, 2018 issued by the Japanese Patent Office in application no. 2014-552048	Machine Translation
	101.	ROOPASHREE S. DWARAKANATH et al., "Recombinant COS-1 cells express Carcinoscopus rotundicauda Factor C", Biotechnology Letters, April 1997, pp. 357-361, Vol. 19, No. 4	
	102.	Merriam-Webster entry for the term "insoluble", cited in Submission from the opponent dated August 4, 2022 in EP Patent No. 3441466	
	103.	Snapshot of the Wiktionary entry for "+ve" as of October 21, 2012 (1 page total)	
	104.	PubChem entry for "sodium bicarbonate (sodium hydrogencarbonate)", 2022 (1 page total)	
	105.	The Wikipedia entry for "Signal peptide", as of November 23, 2012 (3 pages total)	
	106.	SignalP 6.0, "Signal peptides and their cleavage sites in all domains of life", 2022 (1 page total)	
	107.	"SignalP results for Limulus polyphemus", cited in Submission from the opponent dated September 1, 2022 in European Patent No. 3 441 466	
	108.	"Troubleshooting Western Blotting", Hycult biotech, 2010 (3 pages total)	
	109.	Third Party Observations dated October 10, 2022 submitted in European Application No. 19216609.8 Forwarded by EPO on October 13, 2022	
	110.	XIANGJUN et al., "Role of domains in factor C from Tachypleus tridentatus in neutralization endotoxin", "Accession:2006:239467" File CAPLUS, 2003	

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

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[Page 6 of 6]



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
07/03/2023 01:25:41 PM ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Evelyn Jacob

PATENT CENTER # 62380761

FILING DATE -

CUSTOMER # 23373

FIRST NAMED INVENTOR Hikaru MIZUMURA

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Hui Wauters

Documents

TOTAL DOCUMENTS: 13

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Q289014TrackOneRequest.pdf	1	Track One Request	111 KB
Q289014ContinuationTransmittal.pdf	1	Transmittal of New Application	124 KB
Q289014FeeTransmittal.pdf	1	Fee Worksheet (SB06)	116 KB
Q289014SpecificationasFiled-APP.TEXT.docx	104	Application body structured text document	68 KB

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revise if necessary. The paragraph number appears to be in the incorrect format. Please review and revise if necessary.

Q289014DrawingsasfiledParent.pdf	10	Drawings-only black and white line drawings	1719 KB
Q289014SpecificationasFiled.pdf	104	Auxiliary PDF of Application	1260 KB
Q289014ADS.pdf	6	Application Data Sheet	280 KB

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

FEE TRANSMITTAL		Complete if Known	
		Application Number	Not Yet Assigned
		Filing Date	July 3, 2023
☐ Applicant asserts small entity status. See 37 CFR 1.27.		First Named Inventor	Hikaru MIZUMURA
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be attached or have been submitted previously.		Examiner Name	DAVID J STEADMAN (Parent)
		Art Unit	1656 (Parent)
TOTAL AMOUNT OF PAYMENT	(\$6,020	Docket No.	Q289014

METHOD OF PAYMENT (check all that apply)	
☐ Check ☒ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____	
☐ Deposit Account Deposit Account Number: <u>19-4880</u> Deposit Account Name: <u>Sughrue Mion, PLLC</u>	
For the above-identified deposit account, the Director is hereby authorized to (check all that apply):	
☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, except for the Issue Fee and the Publication Fee ☐ Charge fee(s) indicated below, except for the filing fee, the Issue Fee and the Publication Fee ☒ Charge any additional fee(s), except for the Issue Fee and the Publication Fee, or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayment of fees(s)	

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)										
	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	U(\$)	S(\$)	M(\$)	U(\$)	S(\$)	M(\$)	U(\$)	S(\$)	M(\$)	Fees Paid (\$)
Utility	320	128*	64	700	280	140	800	320	160	1,820
Design	220	88	44	160	64	32	640	256	128	
Plant	220	88	44	440	176	88	660	264	132	
Reissue	320	128	64	700	280	140	2320	928	464	
Provisional	300	120	60	0	0	0	0	0	0	

* The \$128 small entity status filing fee for a utility application is further reduced to \$64 for a small entity status applicant who files the application via Patent Center or EFS-Web.

2. EXCESS CLAIM FEES			
Fee Description	Undiscounted Fee (\$)	Small Entity Fee (\$)	Micro Entity Fee (\$)
Each claim over 20 (including Reissues)	100	40	20
Each independent claim over 3 (including Reissues)	480	192	96
Multiple dependent claims	860	344	172
Total Claims	Fee (\$)	Fee (\$)	Fee (\$)
10 - 20 or HP = 0 X 100 = 0			
HP = highest number of total claims paid for, if greater than 20.			
Indep. Claims	Fee (\$)	Fee (\$)	Fee (\$)
1 - 3 or HP = 0 X 480 = 0			
HP = highest number of independent claims paid for, if greater than 3.			

3. APPLICATION SIZE FEE				
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$420 (\$168 for small entity)(\$84 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee(\$)	Fee Paid (\$)
114	- 100 = 14	/ 50 = 1 (round up to a whole number) x	420	= 420
114 * .75	86 - 100 = 0	/ 50 = 0 EFS Fee Discount	420	= 0

4. OTHER FEE(S)		Fees Paid (\$)
Non-English Specification, \$140 fee (\$56 for small entity or \$28 for micro entity)		
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, \$400 fee (\$200 small or micro entity)		
Other (e.g., late filing surcharge): <u>Track One Request</u>		4200

SUBMITTED BY			
Signature	/Hui C. Wauters/	Registration No. (Attorney/Agent)	57,426
Name (Print/Type)	Hui C. Wauters	Telephone:	202.293.7060
		Date:	July 3, 2023

**CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION
UNDER 37 CFR 1.102(e) (Page 1 of 1)**

First Named Inventor:	Hikaru MIZUMURA	Nonprovisional Application Number:	Not Yet Assigned	
Filing Date:	July 3, 2023	Confirmation Number:	Not Yet Assigned	Docket Number: Q289014
Title of Invention:	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN			

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently \$0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

–OR–

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, or the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

- i. A request for continued examination has been filed with, or prior to, this form.
- ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
- iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
- iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
- v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature	/Hui C. Wauters/	Date	July 3, 2023
Name (Print/Typed)	Hui C. Wauters	Practitioner Registration Number	57,426
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*			
☒ *Total of <u>1</u> forms are submitted.			



UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #
18/346,420

RECEIPT DATE / TIME
07/03/2023 01:25:41 PM ET

ATTORNEY DOCKET #
Q289014

Title of Invention

NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 6959

FILED BY Evelyn Jacob

PATENT CENTER # 62380761

AUTHORIZED BY Hui Wauters

CUSTOMER # 23373

FILING DATE -

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Hikaru MIZUMURA

Payment Information

PAYMENT METHOD
CARD / 5804

PAYMENT TRANSACTION ID
E202373D27587111

PAYMENT AUTHORIZED BY
Evelyn Jacob

PRE-AUTHORIZED ACCOUNT
194880

PRE-AUTHORIZED CATEGORY
37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees); 37 CFR 1.19 (Document supply fees); 37 CFR 1.21 (Miscellaneous fees and charges)

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	140.00	1	140.00
1817	REQUEST FOR PRIORITIZED EXAMINATION	4200.00	1	4200.00
1111	UTILITY PATENT APPL. SEARCH FEE	700.00	1	700.00
1011	BASIC FILING FEE - UTILITY (PAPER FILING ALSO REQUIRES NON-	320.00	1	320.00

ELECTRONIC FILING FEE UNDER
1.16(T))

1311	PATENT APPL. EXAMINATION FEE	800.00	1	800.00
TOTAL AMOUNT:				\$6,160.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

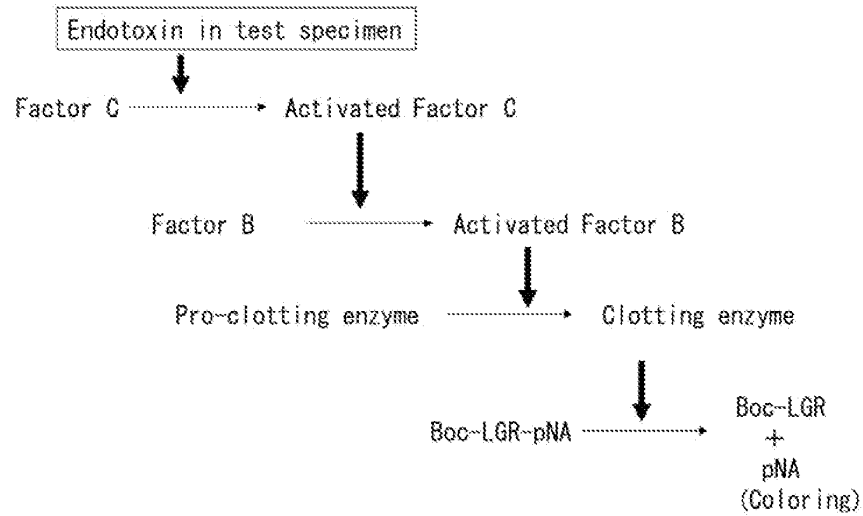
New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

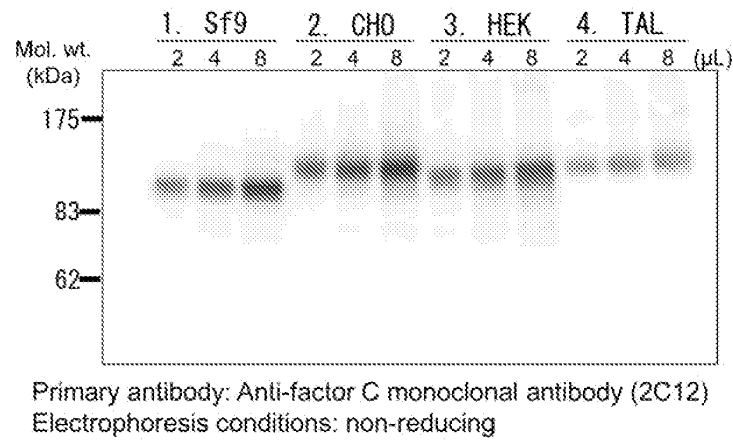
TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS			
NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.			
Application Number	Not Yet Assigned		
Filing Date	July 3, 2023		
First Named Inventor	Hikaru MIZUMURA		
Title	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		
Art Unit	1656 (Parent)		
Examiner Name	DAVID J STEADMAN (Parent)		
Attorney Docket Number	Q289014		
SIGNATURE of Applicant or Patent Practitioner			
Signature	/Hui C. Wauters/	Date (Optional)	July 3, 2023
Name	Hui C. Wauters	Registration Number	57,426
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			
NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.			
☒ *Total of <u>1</u> forms are submitted.			

Figures

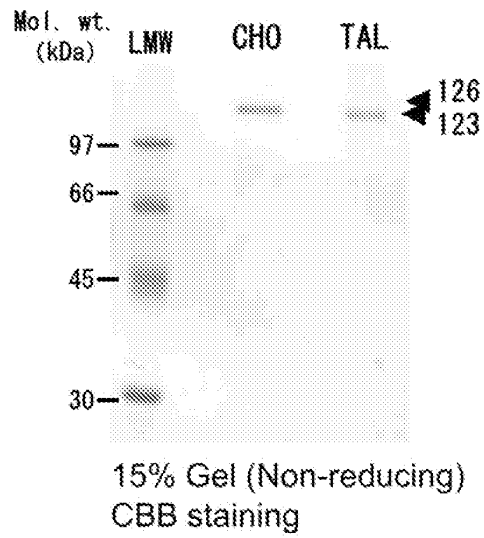
[Fig. 1]



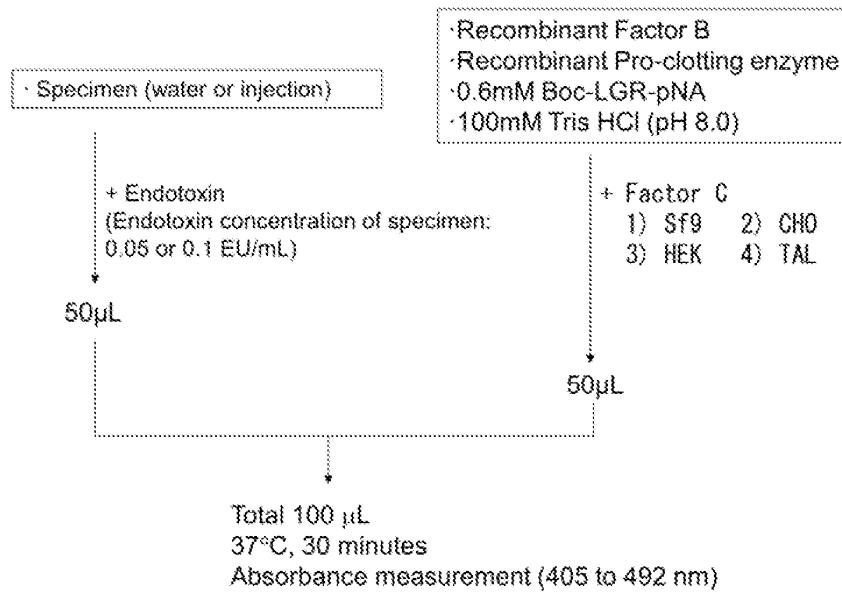
[Fig. 2]



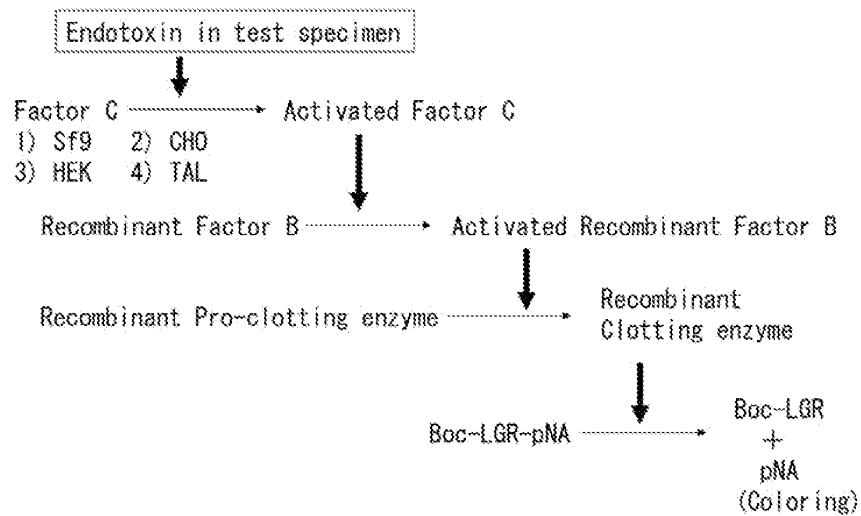
[Fig. 3]



[Fig. 4]



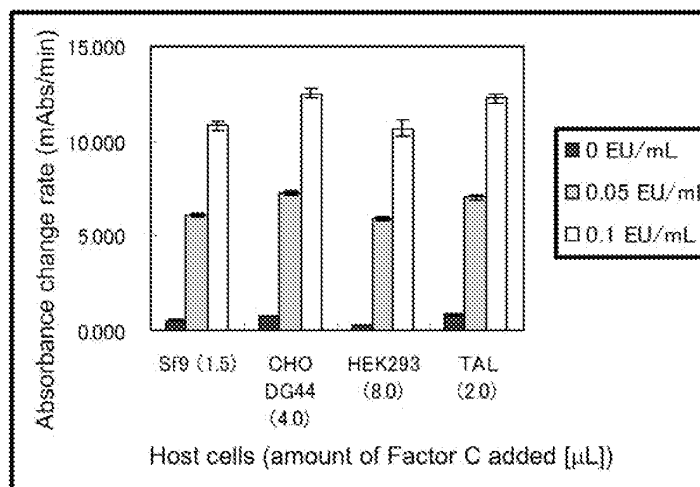
[Fig. 5]



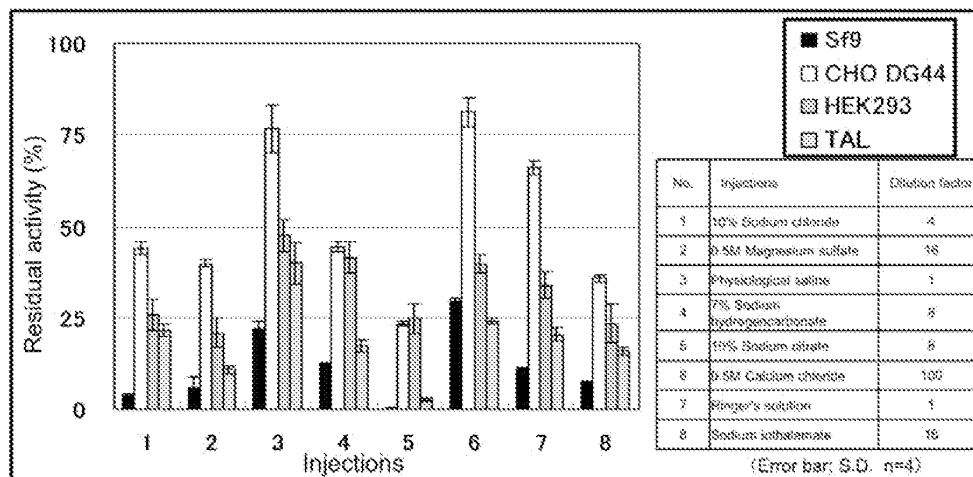
[Fig. 6]

Host cells for Factor C	Amount of Factor C added (μ L)	Endotoxin concentration (EU/mL)	Absorbance change rate (av. $\pm$ S.D.) (mAbs/min)
Sf9	1.5	0	0.520 $\pm$ 0.015
		0.05	6.095 $\pm$ 0.108
		0.1	10.795 $\pm$ 0.246
CHO DG44	4	0	0.770 $\pm$ 0.021
		0.05	7.260 $\pm$ 0.087
		0.1	12.510 $\pm$ 0.255
HEK293	8	0	0.300 $\pm$ 0.017
		0.05	5.885 $\pm$ 0.125
		0.1	10.685 $\pm$ 0.417
TAL	2	0	0.840 $\pm$ 0.055
		0.05	7.030 $\pm$ 0.140
		0.1	12.270 $\pm$ 0.255

n=3



[Fig. 7]



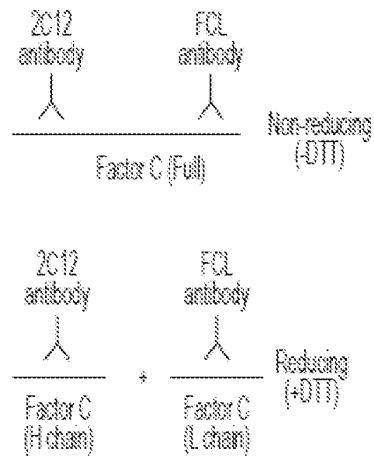
[Fig. 8]

TtFC	MVLASFLVSQLVLGILAQMRPVQSRGVDLGLCDETRFECKCGDPGYVFNVPKQCTYFY	
834CrID4	MVLASFLVSQLVLGILAQMRPVQSKGVDLGLCDETRFECKCGDPGYVFNIPVKQCTYFY	
	*****[***]*****[*****]*****[***]*****	60
TtFC	RWRPYCKPCDDLEAKDIPCXYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ	
834CrID4	RWRPYCKPCDDLEAKDIPCXYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ	
	*****	120
TtFC	RTGACTCRDRYEGAHCEILKGCPLLPDSQVQEVNPPDNPTIDYSCSPGFKLKGVARI	
834CrID4	RTGACACRDRYEGVHCEILKGCPLLPDSQVQEVNPPDNPTIDYSCSPGFKLKGMARI	
	*****[*****]*****[*****]*****[***]	180
TtFC	SCLPNGQWSSFPKCI RECAKVSSPEHGKVNAPSGNMIEGATLRFSCDSPYYLIGGETLT	
834CrID4	SCLPNGQWSNFPPKCI RECAMVSSPEHGKVNALSGDMIEGATLRFSCDSPYYLIGGETLT	
	*****[*****]*****[*****]*****[*****]	240
TtFC	CQNGQWSGQIPQCKKL VFCPDLDPVNHAEHQVKIGVEQKYGGFPQGTEVTTYCSGNYFL	
834CrID4	CQNGQWNGQIPQCKNLVFCPDLDPVNHAEHKVKIGVEQKYGGFPQGTEVTTYCSGNYFL	
	*****[*****]*****[*****]*****[*****]	300
TtFC	MGFNTLKCNPDGSGWSGSPSCVKVADREVDGDSKAVDFLDDVGEPVRIHCPAGCSL TAGT	
834CrID4	MGFDTLKCNPDGSGWSGSPSCVKVADREVDGDSKAVDFLDDVGEPVRIHCPAGCSL TAGT	
	*****[*****]*****[*****]*****[*****]	360
TtFC	VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVNNGPYSDFLGSDNLGKSEELKSLARS	
834CrID4	VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVNNGPYSDFLGSDNLGKSEELKSLARS	
	*****	420
TtFC	FRFDYVSSSTAGRSGCPDGNFEVEENCYVVTSKQRAWERAQGVCTNMAARLAVLDKDLIP	
834CrID4	FRFDYVRSSTAGKSGCPDGNFEVDENCYVVTSKQRAWERAQGVCTNMAARLAVLDKDVIP	
	*****[*****]*****[*****]*****[*****]	480
TtFC	SSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLNDNLTFWASGEPGNETNCVY	
834CrID4	NSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLNDNLTFWASGEPGNETNCVY	
	[*****]*****[*****]*****[*****]	540
TtFC	LDIRDQLQPVWTKSCFQPSFACMMDSRNLKAKCDDPGPLENGHATLHGQSIDGFYAG	
834CrID4	MDIQDQLQSVWTKSCFQPSFACMMDSRNLKAKCDDPGSLENGHATLHGQSIDGFYAG	
	[*****]*****[*****]*****[*****]	600
TtFC	SSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKVITCQNPVPVSYGSVEIKPPSRTN	
834CrID4	SSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKVITCQNPVPVSYGSVEIKPPSRTN	
	*****	660

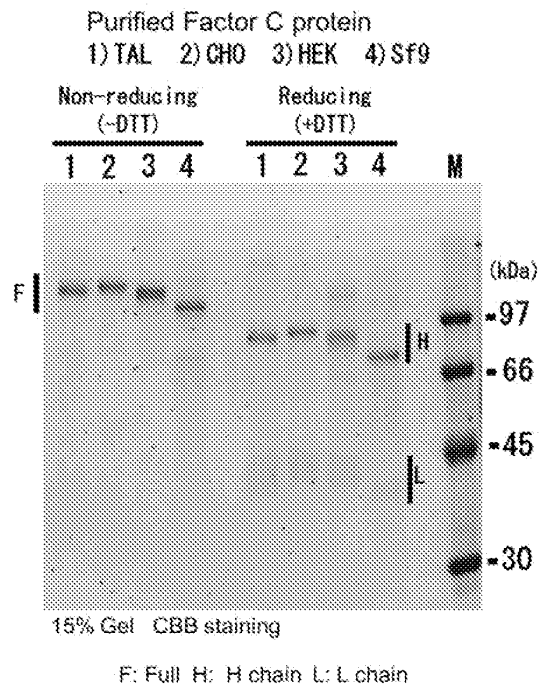
[Fig. 9]

TtFC	SISRVGSPFLRLPRLPLPLARAAPPPKPRSSQPSVDLASKVKLPEGHYRVGSRAIYTC	
834CrID4	SISRVGSPFLRLPRLPLPLARAAPPPKPRSSQPSVDLASKVKLPEGHYRVGSRAIYTC	
	*****	720
TtFC	ESRYYELLGSQGRRCDSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIGQWPWQAGI	
834CrID4	ESRYYELLGSQGRRCDSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIGQWPWQAGI	
	*****	780
TtFC	SRWLADHNMWFQCGGSLNEKWIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDDSROD	
834CrID4	SRWLADHNMWFQCGGSLNEKWIVTAAHCVTYSATAEIIDPNQFKMYLGKYYRDDSROD	
	*****	840
TtFC	DYVQVREALEIHVNPNYDPGNLNFIDIALIQLKTPVILTTRVQPICLPTDITTREHLKEGT	
834CrID4	DYVQVREALEIHVNPNYDPGNLNFIDIALIQLKTPVILTTRVQPICLPTDITTREHLKEGT	
	*****	900
TtFC	LAVVTGWGLNENNTYSEMIQQAVLPVVAASTCEEYKEADLPLTVTENMFCAGYKKGRYD	
834CrID4	LAVVTGWGLNENNTYSEMIQQAVLPVVAASTCEEYKEADLPLTVTENMFCAGYKKGRYD	
	*****	960
TtFC	ACSGDSGGPLVFADDSRTERRWVLEGI VSWGSPSGCGKANQYGGFTKVNVL SWIRQFI	
834CrID4	ACSGDSGGPLVFADDSRTERRWVLEGI VSWGSPSGCGKANQYGGFTKVNVL SWIRQFI	
	*****	1019

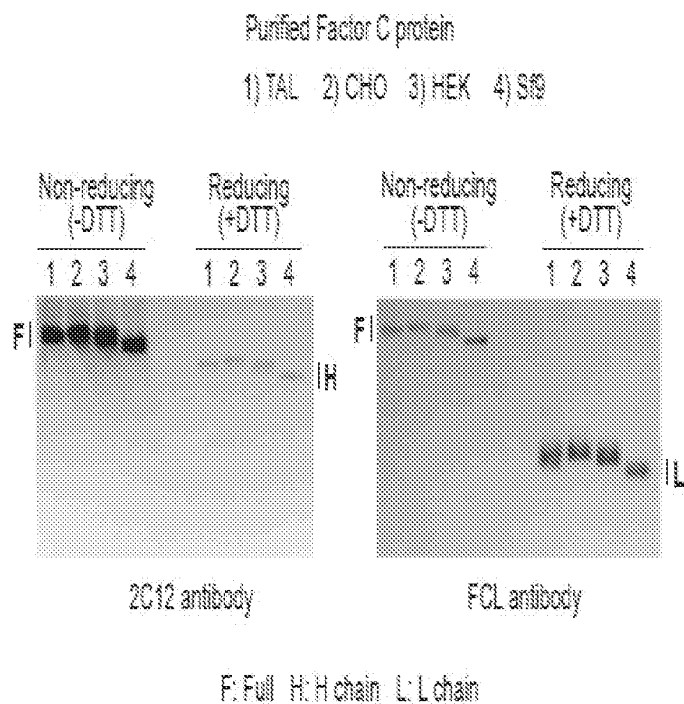
[Fig. 10]



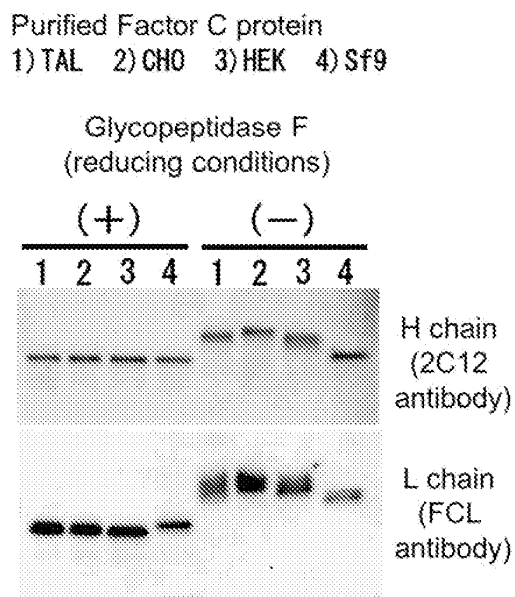
[Fig. 11]



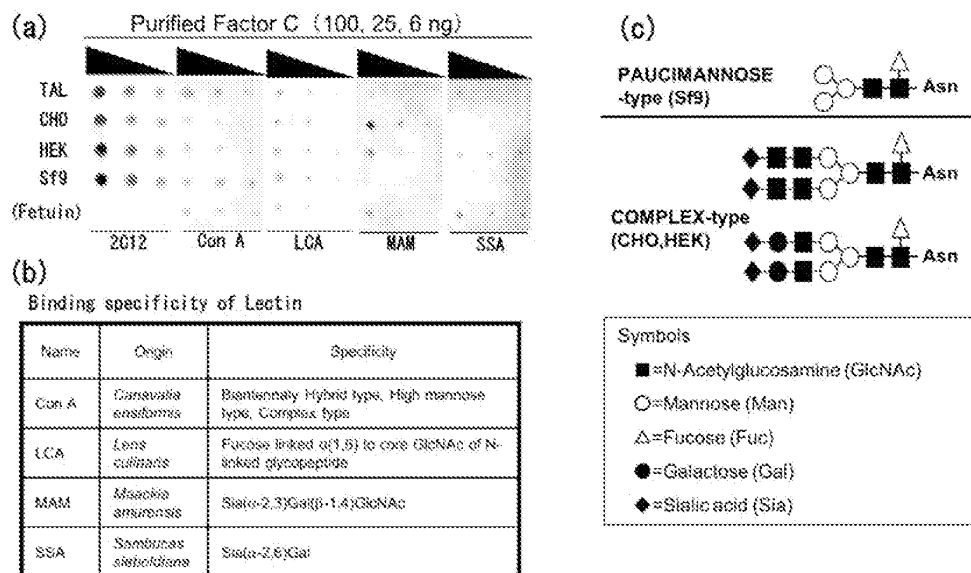
[Fig. 12]



[Fig. 13]



[Fig. 14]



ASSIGNMENT WITH DECLARATION FOR PATENT APPLICATION (37 CFR 1.63)

特許出願宣言書付き譲渡書 (37 CFR 1.63)

Japanese Language Assignment with Declaration

下記に署名した発明者であり、以下に譲渡人と称される私（我々）は、下記の出願に記載された改良に関する発明をし、

の（譲受人）は、本件出願および本件発明、ならびに当該出願から得られるいかなる米国特許に関する全ての権利、権原、および利益の取得を要求します。

よって、価値ある対価の受領をここに確認し、

上で譲渡人として指名された私（我々）は、米国における本件出願およびその発明開示、全ての分割出願および継続出願、これら出願について許可された米国特許証、全ての再発行特許、ならびに全ての外国出願に関する全ての権利（改正後のパリ条約（1883年）に基づく優先権、ならびに 35 USC 119 に基づき優先権を主張する権利と過去分の損害賠償請求権を含む）、権原、および利益を、上で指定した譲受人、その承継人ないし権利承継者に売却、譲渡、移転するとともに、私（我々）は米国特許商標庁長官に対し、本件出願に記載の発明に対する特許証を、譲受人、その承継人ないし権利承継者に発行することを要請し、また、私（我々）は、譲受人が本件発明に係る外国特許出願を行うことに同意し、これ以上の対価なしに、譲受人の求めにより、本件米国・外国特許出願に関して譲受人が必要とみなす全ての書類を作成します。

（公証人による証証は不要であるが、これらの書類は、35 USC 261 に基づく推定された証拠となる）

私は、下記に記載の発明者として、以下の通り宣言します。

本宣言付き譲渡書は、以下に關します。

☐ 添付の出願書、または、

☐ 年 月 日に提出された米国出願あるいは PCT 国際出願番号（確認番号）

本件出願の名称は、以下の通りです。

Whereas, I/We, the undersigned inventor(s) hereinafter called assignor(s), have invented certain improvements described in the application identified below; and

Whereas, SEIKAGAKU CORPORATION of 6-1, Marunouchi 1-chome, Chiyoda-ku, Tokyo 100-0002 Japan, (assignee), desires to acquire the entire right, title, and interest in the application and invention, and to any United States patents to be obtained therefor;

Now therefore, for valuable consideration, receipt whereof is hereby acknowledged,

I/We, the above named assignor(s), hereby sell, assign and transfer to the above named assignee, its successors and assigns, the entire right, title and interest in the application and the invention disclosed therein for the United States of America, including all divisions, and continuations thereof, and all Letters Patent of the United States that may be granted thereon, and all reissues thereof, and all countries foreign thereto, including rights of priority under the International Convention of Paris (1883) as amended, including the right to claim priority under 35 USC 119 and the right to sue for past damages, and I/we request the Director of the U.S. Patent and Trademark Office to issue any Letters Patent granted upon the invention set forth in the application to the assignee, its successors and assigns; and I/we hereby agree that the assignee may apply for foreign Letters Patent on the invention and I/we will execute without further consideration all papers deemed necessary by the assignee in connection with the United States and foreign applications when called upon to do so by the assignee.

(Legalization not required for recording but is prima facie evidence of execution under 35 USC 261)

As the below named inventor, I hereby declare that:

This assignment with declaration is directed to:

☐ The attached application, or

☒ United States Application or PCT International Application Number PCT/JP2012/083082 filed on December 10, 2012 (Confirmation No.)

The application is entitled:

Novel Recombinant Factor C and Method for Producing the Same, and Method for Measuring Endotoxin

Japanese Language Assignment with Declaration

上記に明示された出願は、私が作成した、または作成を許可したものです。

私は、本件出願において請求項に記載された発明の本来の発明者、または、本来の共同発明者であると信じています。

私は、本宣誓付き譲渡書の提出に関わる本件出願の内容を検討し、理解しています。

私は、37 CFR 1.56 に定義されるように、自身が知る全ての特許性に関する重要な情報を、米国特許商標庁に開示する義務があることを認識しています。

私は、本宣誓付き譲渡書において故意に虚偽の供述を行った場合、18 USC 1001 に基づき、罰金あるいは 5 年以下の懲役、または両方による処罰の対象となることを認識しています。

The above identified application was made or was authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I have reviewed and understand the contents of the application for which this assignment with declaration is being submitted.

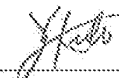
I am aware of the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby acknowledge that any willful false statement made in this assignment with declaration is punishable under 18 USC 1001 by fine or imprisonment of not more than five (5) years, or both.

STATEMENT OF ACCURATE TRANSLATION IN ACCORDANCE WITH 37 CFR 1.69(b):

The assignment with declaration is an accurate translation of the corresponding English language assignment with declaration.

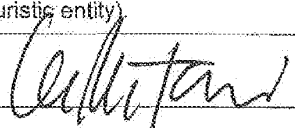
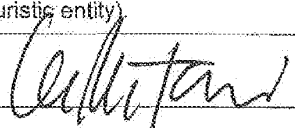
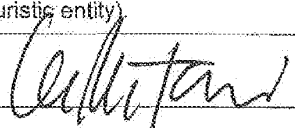
Signature



Date

March 11, 2014

NAME OF SOLE OR FIRST INVENTOR: 唯一あるいは第一の発明者名		
Given Name (first and middle [if any]) 名 (名およびミドルネーム[該当する場合])	Family Name or Surname 姓	
Hikaru	MIZUMURA	
Inventor's signature 発明者の署名	Date 日付	May. 22. 2015
Residence: 住所: Tokyo, Japan		
Mailing Address: 郵送先: c/o SEIKAGAKU CORPORATION Central Research Laboratories, 1253, Tateno 3-chome, Higashiyamato-shi, Tokyo 207-0021 Japan		
NAME OF SECOND INVENTOR: 第二の発明者名:		
Given Name (first and middle [if any]) 名 (名およびミドルネーム[該当する場合])	Family Name or Surname 姓	
Toshio	ODA	
Inventor's signature 発明者の署名	Date 日付	May. 22. 2015
Residence: 住所: Tokyo, Japan		
Mailing Address: 郵送先: c/o SEIKAGAKU CORPORATION Central Research Laboratories, 1253, Tateno 3-chome, Higashiyamato-shi, Tokyo 207-0021 Japan		
NAME OF THIRD INVENTOR: 第三の発明者名:		
Given Name (first and middle [if any]) 名 (名およびミドルネーム[該当する場合])	Family Name or Surname 姓	
Shun-ichiro	KAWABATA	
Inventor's signature 発明者の署名	Date 日付	May. 27. 2015
Residence: 住所: Fukuoka, Japan		
Mailing Address: 郵送先: c/o KYUSHU UNIVERSITY, 6-10-1 Hakozaki, Higashi-ku, Fukuoka-shi, Fukuoka 812-8581 Japan		

POWER OF ATTORNEY BY APPLICANT															
<p>I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.</p> <p>I hereby appoint:</p> <p style="padding-left: 40px;">Patent Practitioner(s) associated with the following Customer Number:</p> <div style="text-align: center; margin: 10px 0;"> <small>WASHINGTON OFFICE</small> 23373 <small>CUSTOMER NUMBER</small> </div> <p>as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (Form PTO/AIA/82A).</p>															
<p>Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:</p> <p style="padding-left: 40px;">The address associated with the above-mentioned Customer Number.</p>															
<p>I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):</p> <div style="border: 1px solid black; padding: 2px; margin: 5px 0;">SEIKAGAKU CORPORATION</div> <div style="margin-top: 10px;"> ☐ Inventor or Joint Inventor (title not required below) ☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below) ☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity) ☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a Petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)(provide signer's title if applicant is a juristic entity) </div>															
<p style="text-align: center;">SIGNATURE of Applicant for Patent</p> <p>The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity)</p> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 10px;"> <tr> <td style="width: 20%; padding: 5px;">Signature</td> <td style="width: 40%; padding: 5px;"></td> <td style="width: 20%; padding: 5px;">Date (Optional)</td> <td style="width: 20%; padding: 5px;">June 10, 2015</td> </tr> <tr> <td style="padding: 5px;">Name</td> <td colspan="3" style="padding: 5px;">Ken MIZUTANI</td> </tr> <tr> <td style="padding: 5px;">Title</td> <td colspan="3" style="padding: 5px;">President</td> </tr> </table>				Signature		Date (Optional)	June 10, 2015	Name	Ken MIZUTANI			Title	President		
Signature		Date (Optional)	June 10, 2015												
Name	Ken MIZUTANI														
Title	President														
<p>NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.</p>															
<p>☒ Total of <u>1</u> forms are submitted.</p>															

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1				
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
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Postal Code	207-0021	Country	JP	
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Prefix	Given Name	Middle Name	Family Name	Suffix
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Postal Code	207-0021	Country	JP	
Inventor 3				
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		

	Shun-ichiro		KAWABATA	
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service				
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Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).	
☐ An Address is being provided for the correspondence information of this application.	
Customer Number	23373
Email Address	sughrue@sughrue.com

Application Information:

Title of the Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		
Attorney Docket Number	Q289014	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

Filing By Reference:

<p>Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").</p> <p>For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).</p>		
Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.			
Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	23373		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.					
When referring to the current application, please leave the "Application Number" field blank.					
Prior Application Status	Pending				
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
	Continuation of	17/554,128	2021-12-17		
Prior Application Status	Patented				
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
17/554,128	Continuation of	16/399,386	2019-04-30	11,236,318	2022-02-01
Prior Application Status	Patented				
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16/399,386	Continuation of	15/983,725	2018-05-18	10,982,202	2021-04-20
Prior Application Status	Patented				
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/983,725	Continuation of	14/650,767	2015-06-09	10,144,923	2018-12-04
Prior Application Status	Pending				
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		
14/650,767	a 371 of international	PCT/JP2013/083082	2013-12-10

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).			
Application Number	Country	Filing Date (YYYY-MM-DD)	Access Code (if applicable)
2012-269840	JP	2012-12-10	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization or Opt-Out of Authorization to Permit Access:

<p>When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).</p> <p>Should applicant choose not to provide an authorization identified in subsection 1 below, applicant <u>must opt-out</u> of the authorization by checking the corresponding box A or B or both in subsection 2 below.</p> <p>NOTE: This section of the Application Data Sheet is <u>ONLY</u> reviewed and processed with the <u>INITIAL</u> filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.</p>
<p>1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)</p> <p>A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby <u>grants the USPTO authority</u> to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).</p> <p>B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby <u>grants the USPTO authority</u> to provide the EPO access to the bibliographic data and search</p>

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		

results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

- ☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.
- ☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant 1			
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
☒ Assignee	☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor	
☐ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor:			
If the Applicant is an Organization check here.		☒	
Organization Name	SEIKAGAKU CORPORATION		
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Country	JP	Postal Code	100-0005
Phone Number		Fax Number	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	Q289014
		Application Number	Not Yet Assigned
Title of Invention	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		

Email Address	
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Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Assignee 1			
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.			
If the Assignee or Non-Applicant Assignee is an Organization check here.			☒
Organization Name	SEIKAGAKU CORPORATION		
Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	6-1, Marunouchi 1-chome, Chiyoda-ku		
Address 2			
City	Tokyo	State/Province	
Country	JP	Postal Code	100-0005
Phone Number		Fax Number	
Email Address			

Signature:

<p>NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the <u>INITIAL</u> filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).</p> <p>This Application Data Sheet <u>must</u> be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, <u>all</u> joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of <u>all</u> joint inventor-applicants.</p> <p>See 37 CFR 1.4(d) for the manner of making signatures and certifications.</p>			
Signature	/Hui C. Wauters/	Date (YYYY-MM-DD)	2023-07-03
Name	Hui C. Wauters	Registration Number	57,426

Novel Recombinant Factor C and Method for Producing the Same,
and Method for Measuring Endotoxin

Cross-Reference To Related Applications

This is a Continuation of U.S. Patent Application No. 17/554,128 filed December 17, 2021, which is a Continuation of U.S. Patent Application No. 16/399,386 filed April 30, 2019, now U.S. Patent No. 11,236,318, which is a Continuation of U.S. Patent Application No. 15/983,725 filed May 18, 2018, now U.S. Patent No. 10,982,202, which is a continuation of U.S. Patent Application No. 14/650,767 filed June 9, 2015, now U.S. Patent No. 10,144,923, which is National Stage of International Application No. PCT/JP2013/083082 filed December 10, 2013, claiming priority based on Japanese Patent Application No. 2012-269840 filed December 10, 2012, the contents of all of which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

The content of the electronically submitted sequence listing, file name: Q289014_sequence listing as filed.XML; size: 23,105 bytes; and date of creation: June 29, 2023, filed herewith, is incorporated herein by reference in its entirety.

Technical Field

[0001]

The present invention relates to a novel recombinant Factor C, to a method for producing the recombinant Factor C, and to a method for measuring endotoxin.

Background Art

[0002]

Endotoxin is a lipopolysaccharide which is present in the outer membrane of Gram-negative bacteria and is known as a strong pyrogen. It is also known that even a very small amount of endotoxin causes various pathological conditions via bacterial infection. Such conditions include not only fever but also release of an inflammatory cytokine concomitant with activation of macrophages, induction of endotoxin shock, and the like. Therefore, detection of endotoxin is essential in pharmaceutical products such as an injection, water, medical devices, and the like. From another aspect, endotoxin is a conceivable main cause for a shock involved in an infection with a Gram-negative bacterium. Thus, the presence of infection and a therapeutic effect can be determined through a blood endotoxin analysis.

[0003]

Meanwhile, it has been known that the American horseshoe crab (*Limulus polyphemus*) undergoes blood clotting when it is infected with a Gram-negative bacterium. This phenomenon has been conventionally employed for the detection

of endotoxin.

[0004]

Specifically, there is known a method for measuring endotoxin by use of a hematocyte extract of a horseshoe crab (i.e. an amebocyte lysate of a horseshoe crab, hereinafter also referred to simply as a lysate) (see, for example, Non-Patent Document 1). This method is called a "limulus test," which employs a cascade reaction of a variety of proteins present in the lysate, which reaction occurs via contact between endotoxin and the lysate. Fig. 1 shows the scheme of the cascade reaction.

[0005]

When endotoxin comes into contact with the lysate, Factor C, which is a serine protease zymogen present in the lysate, is activated to thereby form activated Factor C. The thus-formed activated Factor C activates Factor B present in the lysate, to thereby form activated Factor B. The thus-formed activated Factor B activates Pro-clotting enzyme present in the lysate, to thereby form a corresponding Clotting enzyme.

[0006]

The Clotting enzyme hydrolyzes a specific site of a coagulogen molecule present in the lysate, thereby coagulin gel is formed, and thus the lysate is coagulated. Thus, endotoxin can be measured through measuring the lysate coagulation reaction.

[0007]

Alternatively, endotoxin may also be measured through coloring reaction between the Clotting enzyme and a synthetic substrate. For example, the Clotting enzyme acts on t-butoxycarbonyl-leucyl-glycyl-arginyl-pNA (Boc-Leu-Gly-Arg-pNA), which is a synthetic substrate, to hydrolyze the amino bonds thereof, and thereby pNA is released. Thus, when the synthetic substrate has been added to the reaction system, endotoxin can be measured through measuring the absorbance (at 405 nm) of the coloring substance (pNA).

[0008]

Furthermore, it is known that a cascade reaction system can be reconstituted by use of Factor C, Factor B, and Pro-clotting enzyme, which are purified from a lysate of the Japanese horseshoe crab (Non-Patent Document 2).

[0009]

However, for using such a lysate, or Factor C, Factor B, and Pro-clotting enzyme purified from the lysate, horseshoe crabs must be caught and blood must be recovered there from. Hence, from the viewpoint of protection of biological resources, difficulty is encountered in supply of such ingredients in an inexhaustible manner. Under such circumstances, there is demand for a technique that can produce these ingredients by genetic engineering, to thereby reconstitute a cascade reaction system.

[0010]

For example, there is known a case where Factor C, Factor B, and Pro-clotting enzyme were expressed in insect

cells as host cells, to thereby reconstitute a cascade reaction system (Patent Documents 1 and 2). However, it has been reported that since the reconstituted system contains sodium chloride, magnesium sulfate, or calcium chloride in the reaction system, the cascade reaction is suppressed (Patent Document 1).

[0011]

Alternatively, there is known a case of using mammalian cells as host cells, wherein Factor C derived from the Singaporean horseshoe crab (*Carcinoscorpius rotundicauda*) was expressed in COS-1, which is a cell line derived from African green monkey kidney cells, as host cells. However, it has been reported that when COS-1 was used as a host, the expressed Factor C was insoluble (Non-Patent Documents 3 and 4). That is, there has never been known a case where functional Factor C is produced using mammalian cells as host cells.

Prior Art Documents

Patent Documents

[0012]

Patent Document 1: WO 2008/004674, pamphlet

Patent Document 2: WO 2012/118226, pamphlet

Non-Patent Documents

[0013]

Non-Patent Document 1: Iwanaga S., Curr. Opin. Immunol. Feb; 5(1): 74-82 (1993)

Non-Patent Document 2: Nakamura T., *et al.*, J. Biochem. Mar; 99(3): 847-57 (1986)

Non-Patent Document 3: Roopashree S. Dwarakanath, *et al.*, Biotechnology letters 19(4): 357-361 (1997)

Non-Patent Document 4: Jing Wang, Bow Ho and Jeak L. Ding, Biotechnology letters 23: 71-76 (2001)

Summary of the Invention

Problems to be Solved by the Invention

[0014]

An object of the present invention is to provide a novel recombinant horseshoe crab Factor C, and a method for producing the recombinant Factor C.

[0015]

Meanwhile, injections to be administered in vivo generally contain a variety of salts (ions). In production of such injections, detection of endotoxin is obligated by the pharmacopoeia of the relevant country. Under such circumstances, in an aspect, another object of the present invention is to provide a reconstituted cascade reaction system which is not susceptible to reaction inhibition even in the presence of a salt (ion).

Means for Solving the Problems

[0016]

The present inventors have found that a recombinant horseshoe crab Factor C can be produced by use of human cells

or Chinese hamster cells as host cells, and that endotoxin can be measured by use of the thus-produced recombinant Factor C while reaction inhibition in the presence of a salt (ion) is mitigated. The present invention has been accomplished on the basis of these findings.

[0017]

Accordingly, the present invention encompasses the following modes.

[1]

A horseshoe crab Factor C having activity of Factor C.

[1.1.1]

The above-described Factor C, which contains sialic acid.

[1.1.2]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid.

[1.1.3]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a native Factor C.

[1.1.4]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell.

[1.1.5]

The above-described Factor C, which exhibits a higher

reactivity in lectin blotting by use of *Maackia amurensis* agglutinin, as compared with a native Factor C.

[1.1.6]

The above-described Factor C, which exhibits a higher reactivity in lectin blotting by use of *Maackia amurensis* agglutinin, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell.

[1.2.1]

The above-described Factor C, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

[1.2.2]

The above-described Factor C, which exhibits a residual activity of 20% or higher in the presence of 21 mM sodium citrate.

[1.2.3]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

[1.2.4]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 52 mM sodium hydrogencarbonate.

[1.2.5]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

[1.2.6]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 214 mM sodium chloride.

[1.2.7]

The above-described Factor C, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

[1.2.8]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 16 mM magnesium sulfate.

[1.2.9]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

[1.2.10]

The above-described Factor C, which exhibits a residual activity of 45% or higher in the presence of 2.5 mM calcium chloride.

[1.2.11]

The above-described Factor C, which exhibits a higher residual activity in the presence of 21 mM sodium citrate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[1.2.12]

The above-described Factor C, which exhibits a higher

residual activity in the presence of 52 mM sodium hydrogencarbonate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[1.2.13]

The above-described Factor C, which exhibits a higher residual activity in the presence of 214 mM sodium chloride, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[1.2.14]

The above-described Factor C, which exhibits a higher residual activity in the presence of 16 mM magnesium sulfate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[1.2.15]

The above-described Factor C, which exhibits a higher residual activity in the presence of 2.5 mM calcium chloride, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[1.3.1]

The Factor C, which is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

[1.3.2]

The Factor C, which is a protein shown in the following (A) or (B):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

[1.4]

The above-described Factor C, which is a recombinant protein.

[1.5.1]

The above-described Factor C, which has a molecular weight of 115 kDa to 140 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.2]

The above-described Factor C, which has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.3]

The above-described Factor C, which has a molecular weight of 120 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.4]

The above-described Factor C, which has a molecular weight of 120 kDa to 128 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.5]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 5 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.6]

The above-described Factor C, which has a molecular weight of 128 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.7]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.8]

The above-described Factor C, which has a molecular weight of 126 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.5.9]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 1 kDa, as measured through SDS-PAGE under a non-reducing condition.

[1.6]

The above-described Factor C, which is water-soluble.

[1.7]

The above-described Factor C, which is a culture supernatant containing Factor C.

[1.8]

The above-described Factor C, which has the following characteristics (1) to (3):

(1) having activity of Factor C;

(2) having a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition; and

(3) exhibiting a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

[2]

A method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

[2.1.1]

The above-described method, wherein the mammalian cell is a mammalian cell other than COS-1.

[2.1.2]

The above-described method, wherein the mammalian cell is a cell of a mammal selected from the group consisting of a primate and a rodent.

[2.1.3]

The above-described method, wherein the mammalian cell is a primate cell.

[2.1.4]

The above-described method, wherein the mammalian cell is a rodent cell.

[2.1.5]

The above-described method, wherein the mammalian cell is a cell of a mammal selected from the group consisting of a primate and a rodent, other than monkey.

[2.1.6]

The above-described method, wherein the mammalian cell is a Chinese hamster cell or a human cell.

[2.1.7]

The above-described method, wherein the mammalian cell is a Chinese hamster cell.

[2.1.8]

The above-described method, wherein the mammalian cell is a human cell.

[2.1.9]

The above-described method, wherein the mammalian cell is CHO or HEK.

[2.1.10]

The above-described method, wherein the mammalian cell is CHO.

[2.1.11]

The above-described method, wherein the mammalian cell is HEK.

[2.1.12]

The above-described method, wherein the mammalian cell

is CHO DG44 or HEK293.

[2.1.13]

The above-described method, wherein the mammalian cell is CHO DG44.

[2.1.14]

The above-described method, wherein the mammalian cell is HEK293.

[2.2.1]

The above-described method, wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

[2.2.2]

The above-described method, wherein the Factor C is a protein shown in the following (A) or (B):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

[3]

A horseshoe crab Factor C producible by the above-described method.

[3.1]

The above-described Factor C, which has activity of Factor C.

[3.2.1]

The above-described Factor C, which contains sialic acid.

[3.2.2]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid.

[3.2.3]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a native Factor C.

[3.2.4]

The above-described Factor C, which contains (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell.

[3.2.5]

The above-described Factor C, which exhibits a higher

reactivity in lectin blotting by use of *Maackia amurensis* agglutinin, as compared with a native Factor C.

[3.2.6]

The above-described Factor C, which exhibits a higher reactivity in lectin blotting by use of *Maackia amurensis* agglutinin, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell.

[3.3.1]

The above-described Factor C, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

[3.3.2]

The above-described Factor C, which exhibits a residual activity of 20% or higher in the presence of 21 mM sodium citrate.

[3.3.3]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

[3.3.4]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 52 mM sodium hydrogencarbonate.

[3.3.5]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

[3.3.6]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 214 mM sodium chloride.

[3.3.7]

The above-described Factor C, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

[3.3.8]

The above-described Factor C, which exhibits a residual activity of 25% or higher in the presence of 16 mM magnesium sulfate.

[3.3.9]

The above-described Factor C, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

[3.3.10]

The above-described Factor C, which exhibits a residual activity of 45% or higher in the presence of 2.5 mM calcium chloride.

[3.3.11]

The above-described Factor C, which exhibits a higher residual activity in the presence of 21 mM sodium citrate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[3.3.12]

The above-described Factor C, which exhibits a higher

residual activity in the presence of 52 mM sodium hydrogencarbonate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[3.3.13]

The above-described Factor C, which exhibits a higher residual activity in the presence of 214 mM sodium chloride, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[3.3.14]

The above-described Factor C, which exhibits a higher residual activity in the presence of 16 mM magnesium sulfate, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[3.3.15]

The above-described Factor C, which exhibits a higher residual activity in the presence of 2.5 mM calcium chloride, as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell and/or a native Factor C.

[3.4]

The above-described Factor C, which is a recombinant protein.

[3.5.1]

The above-described Factor C, which has a molecular weight of 115 kDa to 140 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.2]

The above-described Factor C, which has a molecular

weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.3]

The above-described Factor C, which has a molecular weight of 120 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.4]

The above-described Factor C, which has a molecular weight of 120 kDa to 128 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.5]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 5 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.6]

The above-described Factor C, which has a molecular weight of 128 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.7]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.8]

The above-described Factor C, which has a molecular weight of 126 kDa $\pm$ 2 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.5.9]

The above-described Factor C, which has a molecular weight of 127 kDa $\pm$ 1 kDa, as measured through SDS-PAGE under a non-reducing condition.

[3.6]

The above-described Factor C, which is water-soluble.

[3.7]

The above-described Factor C, which is a culture supernatant containing Factor C.

[4]

An endotoxin assay agent containing the above-described Factor C.

[4.1]

The above-described assay agent, which further contains a horseshoe crab Factor B and a horseshoe crab Pro-clotting enzyme.

[4.2]

The above-described assay agent, wherein the Factor B is a protein shown in the following (E) or (F) and the Pro-clotting enzyme is a protein shown in the following (G) or (H):

(E) a protein comprising the amino acid sequence shown in SEQ ID NO: 6;

(F) a protein comprising the amino acid sequence shown in SEQ ID NO:6 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor B;

(G) a protein comprising the amino acid sequence shown

in SEQ ID NO: 8;

(H) a protein comprising the amino acid sequence shown in SEQ ID NO:8 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Pro-clotting enzyme.

[5]

An endotoxin assay kit comprising the above-described assay agent.

[6]

A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the above-described assay agent and the test specimen, and a step of measuring the progress of a cascade reaction.

[6.1.1]

The above-described method, wherein the test specimen contains an ion.

[6.1.2]

The above-described method, wherein the test specimen contains a cation.

[6.1.3]

The above-described method, wherein the test specimen contains a metal ion.

[6.1.4]

The above-described method, wherein the test specimen contains an alkali metal ion or an alkaline earth metal ion.

[6.1.5]

The above-described method, wherein the test specimen

contains an alkali metal ion.

[6.1.6]

The above-described method, wherein the test specimen contains an alkaline earth metal ion.

[6.2.1]

The above-described method, wherein the test specimen contains one or more kinds of ions selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

[6.2.2]

The above-described method, wherein the test specimen contains sodium ion.

[6.2.3]

The above-described method, wherein the test specimen contains potassium ion.

[6.2.4]

The above-described method, wherein the test specimen contains calcium ion.

[6.2.5]

The above-described method, wherein the test specimen contains magnesium ion.

[6.3.1]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the

assay agent.

[6.3.2]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 2 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.3.3]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 5 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.3.4]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 10 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.3.5]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 20 mM or higher, as a final concentration in

the reaction system after mixing the test specimen with the assay agent.

[6.3.6]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 50 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.3.7]

The above-described method, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 100 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.4]

The above-described method, wherein the test specimen is an injection.

[6.5.1]

The above-described method, wherein the test specimen contains one or more kinds of salts selected from the group consisting of sodium chloride, magnesium sulfate, sodium hydrogencarbonate, sodium citrate, calcium chloride, potassium chloride, sodium iothalamate, calcium disodium edetate, and dihydrogen sodium phosphate.

[6.5.2]

The above-described method, wherein the test specimen contains sodium chloride.

[6.5.3]

The above-described method, wherein the test specimen contains magnesium sulfate.

[6.5.4]

The above-described method, wherein the test specimen contains sodium hydrogencarbonate.

[6.5.5]

The above-described method, wherein the test specimen contains sodium citrate.

[6.5.6]

The above-described method, wherein the test specimen contains calcium chloride.

[6.5.7]

The above-described method, wherein the test specimen contains potassium chloride.

[6.5.8]

The above-described method, wherein the test specimen contains sodium iothalamate.

[6.5.9]

The above-described method, wherein the test specimen contains calcium disodium edetate.

[6.5.10]

The above-described method, wherein the test specimen contains dihydrogen sodium phosphate.

[6.6.1]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.2]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 2 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.3]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 5 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.4]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 10 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.5]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 20 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.6]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 50 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.6.7]

The above-described method, wherein the contained amount of the salt in the test specimen is such an amount that the concentration of the salt derived from the test specimen becomes 100 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

[6.7]

The above-described method, which further comprises a step of adding, to the reaction system, a substrate for detecting the progress of the cascade reaction.

[6.8]

The above-described method, which further comprises a

step of calculating the amount of endotoxin in the test specimen.

Brief Description of the Drawings

[0018]

[Fig. 1] A chart showing a cascade reaction system of the limulus test.

[Fig. 2] A photograph showing the results of western blotting of four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (*Tachypleus tridentatus*), under non-reducing conditions.

[Fig. 3] A photograph (SDS-PAGE) showing the molecular weight of each of a native Factor C (TAL) derived from Japanese horseshoe crab (*Tachypleus tridentatus*) and a recombinant Factor C (CHO).

[Fig. 4] A scheme of measuring the activity of Factor C.

[Fig. 5] A chart showing a reconstituted cascade reaction system.

[Fig. 6] A table and a graph showing the activity of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (*Tachypleus tridentatus*).

[Fig. 7] A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (*Tachypleus tridentatus*).

[Fig. 8] Alignment of Factor C of *Tachypleus*

tridentatus (TtFC; SEQ ID NO: 2) and Factor C of *Carcinoscorpius rotundicauda* (834CrID4; SEQ ID NO: 4) (continued to Fig. 9). Consensus sequences which can undergo N-type sugar chain (N-linked sugar chain) modification are underlined.

[Fig. 9] Alignment of Factor C of *Tachypleus tridentatus* (TtFC; SEQ ID NO: 2) and Factor C of *Carcinoscorpius rotundicauda* (834CrID4; SEQ ID NO: 4) (continued from Fig. 8). Consensus sequences which can undergo N-type sugar chain (N-linked sugar chain) modification are underlined.

[Fig. 10] A schematic representation of reaction sites between Factor C and specific antibodies under reducing and non-reducing conditions.

[Fig. 11] A photograph (SDS-PAGE) showing the molecular weight of the four types of purified Factor C derived from Japanese horseshoe crab (*Tachypleus tridentatus*).

[Fig. 12] A photograph (western blotting) showing the molecular weight of the four types of purified Factor C derived from Japanese horseshoe crab (*Tachypleus tridentatus*).

[Fig. 13] A photograph (western blotting) showing the results of removal of N-type sugar chain, by glycopeptidase F, from the four types of purified Factor C derived from Japanese horseshoe crab (*Tachypleus tridentatus*).

[Fig. 14] (a) A photograph (lectin blotting) showing the results of detection of N-type sugar chain of the four

types of purified Factor C derived from Japanese horseshoe crab (*Tachypileus tridentatus*); (b) a table showing the binding specificity of lectin; and (c) a schematic representation of N-type sugar chain structures of a recombinant Factor C, assumed by the experimental results and reports by documents.

Modes for Carrying Out the Invention

In the present invention, a series of reactions wherein endotoxin activates Factor C to generate active-type Factor C; the active-type Factor C activates Factor B to generate active-type Factor B; and the active-type Factor B activates Pro-clotting enzyme to generate a corresponding Clotting enzyme; may be referred to as "cascade reaction". In the present invention, Factor C, Factor B, and Pro-clotting enzyme may be collectively referred to as "factor". In the present invention, a cascade reaction system constituted by combining the factors may be referred to as "reconstituted system". In such a reconstituted system, an effect of a contaminant contained in an amebocyte lysate of a horseshoe crab, e.g. Factor G, on the cascade reaction can be eliminated.

[0019]

(1) Factor C of the present invention

The Factor C of the present invention is a Factor C derived from a horseshoe crab. The Factor C of the present invention is a recombinant protein. The term "recombinant protein" refers to a protein obtained through introduction of

a gene encoding the protein into a host cell and heterologous expression of the gene. The Factor C of the present invention has activity of Factor C.

[0020]

Examples of the horseshoe crab include *Tachypleus tridentatus* (Japanese horseshoe crab), *Limulus polyphemus* (American horseshoe crab), *Carcinoscorpius rotundicauda* (Southeast Asian (Singaporean) horseshoe crab), and *Tachypleus gigas* (Southeast Asian horseshoe crab). Among them, *Tachypleus tridentatus* (Japanese horseshoe crab) and *Carcinoscorpius rotundicauda* (Southeast Asian (Singaporean) horseshoe crab) are preferred as a Factor C source. Furthermore, among them, *Tachypleus tridentatus* (Japanese horseshoe crab) is more preferred as a Factor C source. The amino acid sequence of the Factor C derived from *Tachypleus tridentatus* is shown in SEQ ID NO: 2. The nucleotide sequence of a gene encoding the Factor C derived from *Tachypleus tridentatus* is shown in SEQ ID NO: 1. The amino acid sequence of the Factor C derived from *Carcinoscorpius rotundicauda* is shown in SEQ ID NO: 4. The nucleotide sequence of a gene encoding the Factor C derived from *Carcinoscorpius rotundicauda* is shown in SEQ ID NO: 3.

[0021]

The Factor C of the present invention may undergo N-type sugar chain (N-linked sugar chain) modification. The term "N-type sugar chain" refers to a sugar chain which is linked to an asparagine residue of a protein. In the Factor

C derived from *Tachypleus tridentatus* and the Factor C derived from *Carcinoscorpius rotundicauda*, the amino acid residues which can undergo N-type sugar chain modification are conserved. Figs. 8 and 9 show alignments and consensus sequences (Asn-Xaa-Ser/Thr, wherein Xaa represents any amino acid residue) which can undergo N-type sugar chain modification of both types of Factor C. Thus, in particular, the Factor C derived from *Carcinoscorpius rotundicauda* is thought to be a suitable Factor C as with the Factor C derived from *Tachypleus tridentatus*.

[0022]

The Factor C of the present invention may contain a sialic acid. Examples of the sialic acid include N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). The expression "containing a sialic acid" refers to the state that a sialic acid is linked to the Factor C of the present invention. The sialic acid may be linked to Factor C directly or indirectly. In one case, a sugar chain containing a sialic acid may be linked to Factor C, whereby the sialic acid may be indirectly linked to Factor C. The sialic acid may be, for example, an (α -2,3)-linked sialic acid, more specifically, an (α -2,3)-linked terminal sialic acid. The term "(α -2,3)-linked sialic acid" refers to a sialic acid linked to a sugar via (α -2,3) glycoside linkage. The term "(α -2,3)-linked terminal sialic acid" refers to a sialic acid which is linked to a sugar via (α -2,3) glycoside linkage and which is located at a terminus of the sugar

chain. An example of the sugar chain is an N-type sugar chain (N-linked sugar chain). The sialic acid may be added to Factor C through, for example, expression of Factor C in a host cell which can add a sialic-acid-containing sugar chain to a protein through modification after translation. Examples of such a host cell include mammalian cells. Sialic acid may be detected through, for example, sugar chain analysis. Sugar chain analysis may be carried out through a known technique. For example, sugar chain analysis can be carried out by using lectin or a sugar-chain-recognizing antibody. Specifically, for example, an (α -2,3)-linked terminal sialic acid can be specifically detected through lectin blotting using *Maackia amurensis* agglutinin (MAM).

[0023]

The Factor C of the present invention may contain sialic acid in a greater amount, as compared with a control Factor C. The expression "containing sialic acid in a greater amount, as compared with a control Factor C" refers to the state that the amount of sialic acid linked to the Factor C of the present invention is greater than the amount of sialic acid linked to the control Factor C, in terms of the amount by mole of sialic acid per molecule of Factor C. For example, the amount of sialic acid linked to the Factor C of the present invention may be 1.1 times or greater, 1.2 times or greater, 1.3 times or greater, 1.5 times or greater, 2.0 times or greater, 2.5 times or greater, or 3.0 times or greater than the amount of sialic acid linked to the control

Factor C, in terms of the amount by mole of sialic acid per molecule of Factor C.

[0024]

Examples of the control Factor C include a recombinant Factor C expressed in insect cell Sf9, and a native Factor C. The expression "Factor C expressed in Sf9 as a host cell" refers to Factor C obtainable through expression of a gene encoding Factor C consisting of the same amino acid sequence as that of the Factor C of the present invention, in SF9 as a host cell. The term "native Factor C" refers to Factor C isolated and purified from a horseshoe crab lysate. The native Factor C may be Factor C isolated and purified from a lysate of a horseshoe crab from which the Factor C of the present invention is derived. That is, specifically, for example, when the Factor C of the present invention is a recombinant Factor C of *Tachypleus tridentatus* or a variant thereof, the native Factor C may be Factor C isolated and purified from a lysate of *Tachypleus tridentatus*.

[0025]

Whether or not "the Factor C of the present invention contains sialic acid in a greater amount, as compared with a control Factor C" may be determined through comparison between the sialic acid amount of the Factor C of the present invention and that of the control Factor C. The amount of sialic acid may be determined through, for example, sugar chain analysis. Sugar chain analysis may be carried out through a known technique. For example, sugar chain analysis

can be carried out by using lectin or a sugar-chain-recognizing antibody. Specifically, for example, an (α -2,3)-linked terminal sialic acid can be specifically quantitated through lectin blotting using *Maackia amurensis* agglutinin (MAM). It can be confirmed that the higher the reactivity in lectin blotting using MAM, the greater the amount of (α -2,3)-linked terminal sialic acid, and that is, the greater amount of (α -2,3)-linked terminal sialic acid the Factor C contains. In other words, the Factor C of the present invention may exhibit higher reactivity, as compared with the control Factor C, in lectin blotting using MAM. The expression "exhibit higher reactivity, as compared with the control Factor C, in lectin blotting using MAM" refers to the state that, when the equiamount of the Factor C of the present invention and the control Factor C are subjected to lectin blotting using MAM, the detection intensity (such as the extent of coloring) of the spot attributed to the Factor C of the present invention is higher than that of the spot attributed to the control Factor C.

[0026]

The Factor C of the present invention may have no His-tag added to the C-terminus. Also, the Factor C of the present invention may have no V5-tag added to the C-terminus. Also, the Factor C of the present invention may have no peptide added to the C-terminus. Also, the Factor C of the present invention may have no peptide added to the N-terminus. Also, the Factor C of the present invention may

have no peptide added to any of the N-terminus and the C-terminus.

[0027]

So long as the Factor C of the present invention has activity of Factor C, the Factor C may be a variant of any of the aforementioned horseshoe crab Factor C proteins (e.g., a protein having the amino acid sequence shown in SEQ ID NO: 2 or 4). Such a variant includes, for example, a homologue and an artificially modified product of the aforementioned Factor C. Notably, the amino acid sequence of the variant is not necessarily found in an actual horseshoe crab. That is, the Factor C "of a horseshoe crab or derived from a horseshoe crab" includes a variant of Factor C found in a horseshoe crab and having an amino acid sequence which is not found in any horseshoe crab.

[0028]

The activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B. Whether "the Factor C of the present invention has activity of Factor C" can be confirmed, for example, by combining the Factor C of the present invention with a suitable Factor B and a suitable Pro-clotting enzyme in the presence of endotoxin, and detecting the progress of the cascade reaction. Specifically, the protein of SEQ ID NO: 6 and the protein of SEQ ID NO: 8 may be used as a suitable Factor B and a suitable Pro-clotting enzyme, respectively. The progress of

the cascade reaction may be determined by use of the below-mentioned substrate for detection.

[0029]

So long as the Factor C of the present invention has activity of Factor C, the Factor C of the present invention may be a protein having the amino acid sequence of the above-mentioned Factor C such as the amino acid sequence shown in SEQ ID NO:2 or 4, but which includes substitution, deletion, insertion, or addition of one or several amino acid residues. Although the meaning of the term "one or several" varies depending on the positions of the amino acid residues in the three-dimensional structure of the protein and the types of the amino acid residues, the term specifically refers to preferably 1 to 20, more preferably 1 to 10, still more preferably 1 to 5, particularly preferably 1 to 3. The substitution, deletion, insertion, or addition of one or several amino acid residues is a conservative variation where the function of the protein is appropriately maintained. A typical conservative variation is conservative substitution. Conservative substitution is a variation among Phe, Trp, and Tyr, in the case where the substitution occurs at an aromatic amino acid residue; a variation among Leu, Ile, and Val, in the case where the substitution occurs at a hydrophobic amino acid residue; a variation between Gln and Asn, in the case where the substitution occurs at a polar amino acid residue; a variation among Lys, Arg, and His, in the case where the substitution occurs at a basic amino acid residue; a

variation between Asp and Glu, in the case where the substitution occurs at an acidic amino acid residue; or a variation between Ser and Thr, in the case where the substitution occurs at an amino acid residue having a hydroxyl group. Specific examples of the substitution which can be recognized as conservative substitution include a substitution of Ala by Ser or Thr; a substitution of Arg by Gln, His, or Lys; a substitution of Asn by Glu, Gln, Lys, His, or Asp; a substitution of Asp by Asn, Glu, or Gln; a substitution of Cys by Ser or Ala; a substitution of Gln by Asn, Glu, Lys, His, Asp, or Arg; a substitution of Glu by Gly, Asn, Gln, Lys, or Asp; a substitution of Gly by Pro; a substitution of His by Asn, Lys, Gln, Arg, or Tyr; a substitution of Ile by Leu, Met, Val, or Phe; a substitution of Leu by Ile, Met, Val, or Phe; a substitution of Lys by Asn, Glu, Gln, His, or Arg; a substitution of Met by Ile, Leu, Val, or Phe; a substitution of Phe by Trp, Tyr, Met, Ile, or Leu; a substitution of Ser by Thr or Ala; a substitution of Thr by Ser or Ala; a substitution of Trp by Phe or Tyr; a substitution of Tyr by His, Phe, or Trp; and a substitution of Val by Met, Ile, or Leu. The aforementioned substitution, deletion, insertion, addition, or the like of an amino acid residue(s) also encompasses naturally occurring variations such as those based on individual differences between horseshoe crabs and the strain and species of the horseshoe crabs, from which the gene is derived.

[0030]

The Factor C of the present invention may also be a protein which has a homology or identity of 80% or higher, preferably 90% or higher, more preferably 95% or higher, still more preferably 97% or higher, particularly preferably 99% or higher with respect to the entire amino acid sequence of Factor C (e.g., the entire amino acid sequence shown in SEQ ID NO: 2 or 4) and which has activity of Factor C.

[0031]

The gene encoding the Factor C of the present invention is not particularly limited, so long as the gene codes for the aforementioned Factor C of the present invention. The gene encoding the Factor C of the present invention may be a DNA encoding a protein having activity of Factor C, wherein the DNA fragment is hybridized with a probe prepared from a known gene sequence, e.g., with the complementary sequence to the entirety or a part of the nucleotide sequence shown SEQ ID NO: 1 or 3, under stringent conditions. As used herein, the term "stringent conditions" generally refer to the conditions under which a specific hybrid is formed but a non-specific hybrid is not formed. An example of such conditions is conditions under which DNAs having a high homology, e.g., a homology of 80% or higher, preferably 90% or higher, more preferably 95% or higher, still more preferably 97% or higher, particularly preferably 99% or higher, are hybridized with each other, and DNAs having a homology lower than the above level are not hybridized with each other. Another example of the conditions is conditions generally employed in

washing of southern hybridization; i.e., washing once, preferably 2 to 3 times, at temperature and salt concentration corresponding to 60°C, 1×SSC, 0.1% SDS, preferably 60°C, 0.1×SSC, 0.1% SDS, more preferably 68°C, 0.1×SSC, 0.1% SDS.

[0032]

In the gene encoding the Factor C of the present invention, any codon may be substituted by its equivalent codon. For example, the codon combination of the gene encoding the Factor C of the present invention may be modified such that the codon combination is optimized for expression in a mammalian cell. The optimization may be performed through employment of, for example, a conventional contract service. Notably, the gene encoding the Factor C of the present invention may be a variant of a DNA in which the codon combination is optimized for expression in a mammalian cell.

[0033]

The above descriptions about the variants of genes and proteins may also be applied *mutatis mutandis* to Factor B, Pro-clotting enzyme, and gene encoding the same.

[0034]

The Factor C of the present invention may have a molecular weight of 115 kDa to 140 kDa, 115 kDa to 130 kDa, 120 kDa to 130 kDa, or 120 kDa to 128 kDa, as measured through SDS-PAGE under a non-reducing condition. Also, the Factor C of the present invention may have a molecular weight

of 127 kDa $\pm$ 5 kDa, 128 kDa $\pm$ 2 kDa, 127 kDa $\pm$ 2 kDa, 126 kDa $\pm$ 2 kDa, or 127 kDa $\pm$ 1 kDa, as measured through SDS-PAGE under a non-reducing condition. Also, the Factor C of the present invention may have a molecular weight of 128 kDa or 126 kDa, as measured through SDS-PAGE under a non-reducing condition. [0035]

The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. The expression "low susceptibility to inhibition of activity by an ion" refers to the state that the Factor C of the present invention exhibits a high residual activity in the presence of an ion, as compared with a control Factor C. Examples of the control Factor C include a recombinant Factor C expressed in insect cell Sf9 and a native Factor C. The meaning of the expression "exhibiting a high residual activity" is not particularly limited, so long as the residual activity of the Factor C of the present invention is higher than that of the control Factor C. The expression "exhibiting a high residual activity" may refer to, for example, the state that the residual activity of the Factor C of the present invention is 1.1 times or higher, 1.2 times or higher, 1.3 times or higher, 1.5 times or higher, 2.0 times or higher, 2.5 times or higher, or 3.0 times or higher than the residual activity of the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" include such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 21 mM

sodium citrate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 214 mM sodium chloride, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property that the Factor C of the present invention exhibits a higher residual activity in the presence of 2.5 mM calcium chloride, as compared with the control Factor C. Specific examples of "low susceptibility to inhibition of activity by an ion" further include such a property that the Factor C of the present invention exhibits a residual activity of 10% or higher or 20% or higher in the presence of 21 mM sodium citrate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a residual activity of 25% or higher or 35% or higher in the presence of 52 mM sodium hydrogencarbonate, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a residual activity of 25% or higher or 35% or higher in the presence of 214 mM sodium chloride, as compared with the control Factor C; such a property that the Factor C of the present invention exhibits a residual activity of 15% or higher or 25% or

higher in the presence of 16 mM magnesium sulfate, as compared with the control Factor C; and such a property that the Factor C of the present invention exhibits a residual activity of 35% or higher or 45% or higher in the presence of 2.5 mM calcium chloride, as compared with the control Factor C. The Factor C of the present invention may have any one of the above properties singly or two or more of the above properties in combination. The "residual activity" is defined as a relative reactivity (%) observed for a measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to an ion-containing test specimen, with respect to the reactivity observed for another measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to injection water as 100%, in the assay system shown in Fig. 4.

[0036]

The Factor C of the present invention may be water-soluble. As used herein, the term "water-soluble" refers to the state that, when the Factor C of the present invention is expressed in an appropriate host cell, the thus-expressed Factor C is detected in a soluble fraction. The expression "detected in a soluble fraction" may refer to the state that 20% or more, 50% or more, 80% or more, 90% or more, or 95% or more of the expressed Factor C is detected in a soluble fraction.

[0037]

(2) Method for producing the Factor C of the present

invention

The Factor C of the present invention may be produced through, for example, expression in a mammalian cell as a host. That is, the present invention provides a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell. This method may also be referred to as "the production method of the present invention." The Factor C producible through production method of the present invention is an embodiment of the Factor C of the present invention.

[0038]

The mammalian cell is not particularly limited, so long as the cell can functionally express a horseshoe crab Factor C. The term "functional expression" refers to the state that the expressed Factor C exhibits activity of Factor C. Specifically, the mammalian cell may be a cell other than COS-1 cell. The mammalian cell is preferably a cell of a mammal selected from the group consisting of a rodent and a primate. Examples of the rodent include, but are not particularly limited to, Chinese hamster, hamster, mouse, rat, and guinea pig. Of these, Chinese hamster is preferred. Examples of the Chinese hamster cell include Chinese hamster ovary (CHO) cell line. Examples of CHO include CHO DG44 and CHO K1. Examples of the primate include, but are not particularly limited to, human, monkey, and chimpanzee. The primate may be a primate other than monkey. Of these, human is preferred. Examples of the human cell include human

embryo kidney (HEK) cell line. Examples of HEK include HEK293.

[0039]

When the mammalian cell retains a gene encoding Factor C, the cell can express Factor C. The gene encoding Factor C may be retained in the mammalian cell, such that it can be expressed under the control of a promoter which functions in the cell. In the mammalian cell, the gene encoding Factor C, for example, may be present on a vector such as a plasmid which undergoes autonomous replication outside a chromosome, or may be introduced to a chromosome. The technique of introducing the gene encoding Factor C into a mammalian cell is not particularly limited. Examples of the vector which undergoes autonomous replication outside a chromosome include pCA7 (Makoto Takeda, *et al.*, J. Virol. 79(22): 14346-54 (2005)). Alternatively, the gene encoding Factor C may be introduced to a chromosome of a mammalian cell by use of pCI-Neo Vector (product of Promega). The mammalian cell may retain one copy of, or two or more copies of the gene encoding Factor C.

[0040]

The culture conditions under which a mammalian cell is cultured are not particularly limited, so long as the mammalian cell can proliferate. Conditions generally employed in culturing mammalian cells may be employed, after appropriate modification has been performed, if needed. A culture medium which is generally employed in culturing

mammalian cells may be employed as the culture medium in the invention. Specifically, an RPMI 1640 medium (product of Sigma) or a DMEM medium (product of Sigma) may be used. Culturing can be performed, for example, as static culture at 36°C to 38°C under feeding of 5% CO₂.

[0041]

Whether or not Factor C has been functionally expressed can be confirmed by measuring activity of Factor C. Expression of Factor C may also be confirmed by measuring the amount of mRNA formed via transcription of a gene encoding Factor C, or detecting Factor C through western blotting by use of an antibody.

[0042]

The expressed Factor C can be recovered as a solution containing Factor C, and can be used as a component of the endotoxin assay agent of the present invention. The solution containing Factor C may be, for example, a culture broth, a culture supernatant, a disrupted cell extract, a mixture thereof, or the like. Factor C may be used after being purified to a desired extent, or may be used without any purification. In the present invention, even when a cell culture supernatant containing expressed Factor C is used as it is without any purification, satisfactory endotoxin assay performance can be attained. Purification of Factor C may be performed through, for example, a known protein purification technique. Examples of such a technique include ammonium sulfate precipitation, gel filtration chromatography, ion

exchange chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography, and so forth. In the case where a tag such as His-tag has been added to Factor C, the Factor C may be purified through affinity chromatography based on affinity to the tag. A solution containing Factor C may be used after being filtered by means of a filter. For example, a 0.22- μ m filter can be used as the filter.

[0043]

(3) Endotoxin assay agent of the present invention

The Factor C of the present invention may be used in endotoxin assay. That is, the present invention provides an endotoxin assay agent containing the Factor C of the present invention. This endotoxin assay agent may also be referred to as an "endotoxin assay agent of the present invention." The endotoxin assay agent of the present invention may further contain a factor or factors other than Factor C, depending on the mode of endotoxin assay.

[0044]

For example, the Factor C of the present invention may be used in combination with Factor B and Pro-clotting enzyme, for endotoxin assay. In other words, an embodiment of the endotoxin assay agent of the present invention is an endotoxin assay agent containing the Factor C of the present invention, Factor B, and Pro-clotting enzyme. In this case, endotoxin can be measured by detecting Clotting enzyme, which is the final product of the cascade reaction. The Factor B

and the Pro-clotting enzyme contained in the embodiment of the endotoxin assay agent of the present invention may also be referred to as the "Factor B of the present invention" and the "Pro-clotting enzyme of the present invention", respectively.

[0045]

Alternatively, endotoxin can be measured by detecting an intermediate stage of the cascade reaction. In this case, it is sufficient that the endotoxin assay agent of the present invention contains a factor or factors involved in from the start of the cascade reaction to the relevant intermediate stage. Specifically, for example, when endotoxin is measured by detecting the activated Factor C, which is an intermediate of the cascade reaction, it is sufficient that the endotoxin assay agent of the present invention contains Factor C.

[0046]

The Factor B of the present invention is a Factor B derived from a horseshoe crab. The Pro-clotting enzyme of the present invention is a Pro-clotting enzyme derived from a horseshoe crab. Examples of the horseshoe crab include *Tachypleus tridentatus* (Japanese horseshoe crab), *Limulus polyphemus* (American horseshoe crab), *Carcinoscorpius rotundicauda* (Southeast Asian (Singaporean) horseshoe crab), and *Tachypleus gigas* (Southeast Asian horseshoe crab). Among them, *Tachypleus tridentatus* (Japanese horseshoe crab) is preferred as a source of Factor B and Pro-clotting enzyme.

[0047]

The amino acid sequence of the Factor B of *Tachypleus tridentatus* and that of the Pro-clotting enzyme of *Tachypleus tridentatus* are shown in SEQ ID NOs: 6 and 8, respectively. The nucleotide sequence of a gene encoding the Factor B of *Tachypleus tridentatus* and that of a gene encoding the Pro-clotting enzyme of *Tachypleus tridentatus* are shown in SEQ ID NOs: 5 and 7, respectively.

[0048]

The Factor B of the present invention may be a variant of any of the aforementioned horseshoe crab Factor B proteins (e.g., a protein having the amino acid sequence shown in SEQ ID NO: 6), so long as the variant has activity of Factor B. Such a variant includes, for example, a homologue and an artificially modified product of the aforementioned Factor B. The gene encoding the Factor B of the present invention is not particularly limited, so long as the gene codes for the Factor B of the present invention. The above descriptions about the variants of Factor C and the gene encoding Factor C may also be applied *mutatis mutandis* to the variants of Factor B and the gene encoding Factor B.

[0049]

The activity of Factor B refers to such an activity that the Factor B is activated in the presence of activated Factor C to convert Pro-clotting enzyme into the activated form thereof, i.e. the corresponding Clotting enzyme. Whether "the Factor B of the present invention has activity

of Factor B" can be confirmed, for example, by combining the Factor B of the present invention with a suitable Factor C and a suitable Pro-clotting enzyme in the presence of endotoxin, and detecting the progress of the cascade reaction. Specifically, the protein of SEQ ID NO: 2 and the protein of SEQ ID NO: 8 may be used as a suitable Factor C and a suitable Pro-clotting enzyme, respectively. The progress of the cascade reaction may be determined by use of the below-mentioned substrate for detection.

[0050]

The Pro-clotting enzyme of the present invention may be a variant of any of the aforementioned horseshoe crab Pro-clotting enzymes (e.g., a protein having the amino acid sequence shown in SEQ ID NO: 8), so long as the variant has activity of Pro-clotting enzyme. Such a variant includes, for example, a homologue and an artificially modified product of the aforementioned Pro-clotting enzyme. The gene encoding the Pro-clotting enzyme of the present invention is not particularly limited, so long as the gene codes for the Pro-clotting enzyme of the present invention. The above descriptions about the variants of Factor C and the gene encoding Factor C may also be applied *mutatis mutandis* to the variants of Pro-clotting enzyme and the gene encoding Pro-clotting enzyme.

[0051]

The activity of Pro-clotting enzyme is such an activity that the Pro-clotting enzyme is converted into the activated

form thereof, i.e. the corresponding Clotting enzyme, in the presence of activated Factor B, to react with the below-mentioned substrate for detection. The activity of reacting with the detection substrate refers to, for example, an activity of reacting with a coagulogen to induce coagulation, or an activity of reacting with the below-described substrate represented by X-Y-Z (wherein X represents a protection group, Y represents a peptide, and Z represents a dye linked to Y via amide bond), to thereby release dye Z (e.g., an activity of reacting with Boc-Leu-Gly-Arg-pNA, to thereby release pNA). Whether "the Pro-clotting enzyme of the present invention has the activity of Pro-clotting enzyme" can be confirmed, for example, by combining the Pro-clotting enzyme of the present invention with a suitable Factor C and a suitable Factor B in the presence of endotoxin, and detecting the progress of the cascade reaction. Specifically, the protein of SEQ ID NO: 2 and the protein of SEQ ID NO: 6 may be used as a suitable Factor C and a suitable Factor B, respectively. The progress of the cascade reaction may be measured by use of the below-mentioned substrate for detection.

[0052]

So long as the Factor B of the present invention and the Pro-clotting enzyme of the present invention has activity of Factor B and activity of Pro-clotting enzyme, respectively, the Factor B of the present invention and/or the Pro-clotting enzyme of the present invention may have any

peptide or the like added thereto. Examples of such a peptide include tag sequences such as His-tag and V5-tag. Similar to Factor C of the present invention, the Factor B of the present invention and/or the Pro-clotting enzyme of the present invention may have no His-tag added to the C-terminus; may have no V5-tag added to the C-terminus; may have no peptide added to the C-terminus; may have no peptide added to the N-terminus; or may have no peptide added to any of the N-terminus and the C-terminus.

[0053]

In the gene encoding the Factor B of the present invention and/or the gene encoding the Pro-clotting enzyme of the present invention, any codon may be substituted by its equivalent codon. For example, the codon combination of the gene encoding the Factor B of the present invention and/or the gene encoding the Pro-clotting enzyme of the present invention may be modified such that the codon combination is optimized for expression in a host cell. An example of DNA which code for Factor B of SEQ ID NO: 6 and whose codon combination is optimized for expression in an insect cell is a DNA of SEQ ID NO: 9. Notably, the gene encoding the Factor B of the present invention and/or the gene encoding the Pro-clotting enzyme of the present invention may be a variant of a DNA in which the codon combination is optimized for expression in a host cell.

[0054]

The Factor B of the present invention and the Pro-

clotting enzyme of the present invention may each be a native protein or a recombinant protein.

[0055]

A native Factor B and a native Pro-clotting enzyme may each be obtained from the hematocyte extract of the aforementioned various horseshoe crabs. These factors may be used after being purified to a desired extent. Purification may be performed through, for example, a known technique (Nakamura T. *et al.*, J. Biochem. 1986 Mar; 99(3): 847-57).

[0056]

The recombinant Factor B and the recombinant Pro-clotting enzyme may each be obtained through expression in a host cell. The host cell is not particularly limited, so long as a factor of interest can be expressed. For example, a host cell generally employed for heterologous protein expression may be used. Examples of the host cell include insect cells, animal cells, plant cells, yeast cells, and bacterial cells. Among them, an eukaryotic cell is preferred from the viewpoint of modification after translation. Specifically, an insect cell and an animal cell are more preferred.

[0057]

Examples of the animal cells include mammalian cells. In the case where an animal cell such as a mammalian cell is used, the above descriptions about production of the Factor C of the present invention may also be applied *mutatis mutandis* to production of the Factor B and the Pro-clotting enzyme.

[0058]

Examples of the insect cells include Sf9, Sf21, SF+, and High-Five. Of these, Sf9 is preferred as the insect cell.

[0059]

The technique for expressing a factor of interest in an insect cell as a host is not particularly limited, so long as the factor can be expressed. For example, techniques generally employed for heterologous protein expression may be suitably used. For example, a factor of interest can be expressed by infecting an insect cell with a virus into which a gene encoding the factor has been incorporated (the technique being called a "virus method"). Also, for example, a factor of interest can be expressed by incorporating into an insect cell a vector into which a gene encoding the factor has been incorporated, to thereby incorporate the gene into a chromosome of the host cell (the technique being called a "stably expressing cell line method").

[0060]

<Virus method>

The virus employed in the virus method is not particularly limited, so long as an insect cell can be infected with the virus, to thereby express a factor of interest. A virus generally employed for protein expression in insect cells may be suitably used. Examples of such viruses include Baculovirus. Baculovirus is preferably Nucleopolyhedrovirus (NPV). Examples of NPV include

Autographa californica NPV (AcNPV) and *Bombyx mori* NPV (BmNPV). NPV is preferably AcNPV.

[0061]

Incorporation of a nucleic acid into a virus may be performed through a conventional method. For example, incorporation of a nucleic acid into a virus may be performed through homologous recombination by use of a transfer vector. Examples of the transfer vector include pPSC8 (product of Protein Sciences), pFastBac (product of Life technologies corporation), and pVL1393 (product of Pharmingen). Among them, pPSC8 is a preferable transfer vector.

[0062]

An insect cell can be infected, through a conventional technique, with the virus into which a gene encoding a factor of interest has been incorporated, to thereby obtain the insect cell which retain the virus and express the factor.

[0063]

<Stably expressing cell line method>

Through incorporation of a gene encoding a factor of interest into a chromosome of an insect cell, a stably expressing cell line which stably expresses the factor can be established. The method of establishing the stably expressing cell line is not particularly limited, and a conventional technique may be employed. For example, a stably expressing cell line can be established by use of pIZ-V5 (product of Life Technologies Corporation), according to a user's instruction attached thereto.

[0064]

The conditions under which an insect cell is cultured are not particularly limited, so long as the insect cell can proliferate. Conditions generally employed in culturing insect cells may be employed, after appropriate modification has been performed, if needed. A culture medium which is generally employed in culturing insect cells may be employed as the culture medium in the invention. Examples of such a culture medium include a commercially available serum-free medium for culturing insect cells. Specifically, an Sf900III serum-free medium (product of Life Technologies Corporation) or the like may be suitably used. Culturing can be performed, for example, as shaking culture at 27°C to 28°C.

[0065]

Whether or not a factor of interest has been expressed can be confirmed by measuring activity of the factor. Expression of a factor of interest may also be confirmed by measuring the amount of mRNA formed via transcription of a gene encoding the factor, or detecting the factor through western blotting by use of an antibody.

[0066]

The expressed factor can be recovered as a solution containing the factor, and can be used as a component of the endotoxin assay agent of the present invention. The solution containing the factor may be, for example, a culture broth, a culture supernatant, a disrupted cell extract, a mixture thereof, or the like. The factor may be used after being

purified to a desired extent, or may be used without any purification. In the present invention, even when a cell culture supernatant containing the expressed factor is used as it is without any purification, satisfactory endotoxin assay performance can be attained. Purification of the factor may be performed through, for example, a known protein purification technique. Examples of such a technique include ammonium sulfate precipitation, gel filtration chromatography, ion exchange chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography, and so forth. In the case where a tag such as His-tag has been added to the factor, the factor may be purified through affinity chromatography based on affinity to the tag. A solution containing the factor may be used after being filtered by means of a filter. For example, a 0.22- μ m filter can be used as the filter.

[0067]

In the case each factor is produced through the virus method, the virus is preferably removed. The method of removing virus is not particularly limited, and a conventional technique may be employed. For example, the virus may be removed by means of a hollow fiber filtration membrane having a pore size of 500 kDa.

[0068]

In the present invention, the Factor C of the present invention, the Factor B of the present invention, and the Pro-clotting enzyme of the present invention may be

separately expressed in individually established expression cells.

[0069]

The endotoxin assay agent of the present invention may contain, for example, only the Factor C of the present invention. Alternatively, the agent may contain, for example, only the Factor C of the present invention, the Factor B of the present invention, and the Pro-clotting enzyme of the present invention.

[0070]

The endotoxin assay agent of the present invention may further contain a substrate for detecting the progress of the cascade reaction. As used herein, such a substrate may be referred to as a detection substrate.

[0071]

Examples of the detection substrate include coagulogen. Coagulogen is a detection substrate with respect to Clotting enzyme, which is the final product of the cascade reaction. When coagulogen comes into contact with Clotting enzyme, a coagulated product, coagulin, is formed. The progress of the coagulation reaction can be determined by measuring the turbidity of the reaction mixture. Coagulogen can be recovered from a hematocyte extract of a horseshoe crab (i.e., lysate). Since the nucleotide sequence of the gene encoding coagulogen has been determined (Miyata *et al*, Protein Nucleic acid Enzyme, extra issue, No. 29; p. 30-43; 1986), coagulogen can also be produced genetically according

to a conventional technique.

[0072]

The detection substrate may be a synthetic substrate. The synthetic substrate is not particularly limited, so long as it has a property suitable for detection of the cascade reaction. Examples of the "property suitable for detection of the cascade reaction" include a property for detecting the presence of Clotting enzyme and a property for detecting an intermediate stage of the cascade reaction. Examples of the "property for detecting the presence of Clotting enzyme" include a property of coloring by catalytic reaction with the Clotting enzyme and a property of generating fluorescence by catalytic reaction with the Clotting enzyme. Examples of the "property for detecting an intermediate stage of the cascade reaction" include a property of coloring by catalytic reaction of a cascade reaction intermediate such as activated Factor C and a property of generating fluorescence by catalytic reaction of a cascade reaction intermediate such as activated Factor C. Examples of the synthetic substrate include a substrate represented by formula X-Y-Z (wherein X represents a protection group, Y represents a peptide, and Z represents a dye linked to Y via amide bond). When endotoxin is present in the reaction system, an amide linkage Y-Z is cleaved by the catalytic reaction of Clotting enzyme or an intermediate, which is yielded by the cascade reaction, to release the dye Z, whereby coloring occurs or fluorescence is generated. The protective group X is not particularly

limited, and a known protective group for peptide may be suitably used. Examples of such a protective group include a t-butoxycarbonyl group and a benzoyl group. The dye Z is not particularly limited, and it may be a dye detectable under visible light or may be a fluorescent dye. Examples of the dye Z include p-nitroaniline (pNA), 7-methoxycoumarin-4-acetic acid (MCA), 2,4-dinitroaniline (DNP), and Dansyl dyes. Examples of the peptide Y include Leu-Gly-Arg (LGR), Ile-Glu-Gly-Arg (IEGR) (SEQ ID NO: 10), Val-Pro-Arg (VPR), and Asp-Pro-Arg (DPR). A synthetic substrate which reacts with the Clotting enzyme or the intermediate may be appropriately selected as the synthetic substrate in accordance with the mode of endotoxin assay. For example, from the viewpoint of substrate specificity, a substrate comprising LGR as peptide Y can be suitably used for detecting the Clotting enzyme, and a substrate comprising VPR or DPR as peptide Y can be suitably used for detecting activated Factor C. The released dye Z may be determined through a technique in accordance with the property of the target dye.

[0073]

The endotoxin assay agent of the present invention may further contain an additional component other than the relevant factors and a detection substrate, so long as the assay agent can be used for endotoxin assay. The additional component is not particularly limited, and it may be selected in consideration of storage stability, handling easiness, stability of the factors and detection substrate, and so

forth.

[0074]

The endotoxin assay agent of the present invention may be formulated into any preparation such as solid, liquid, or gel. In the formulation process, additives such as an excipient, a binder, a disintegrant, a lubricant, a flavor/corrigent, a diluent, and a solvent, which are generally used in formulation, may be used. The endotoxin assay agent of the present invention as is may be used for endotoxin assay. Alternatively, the assay agent may be used for endotoxin assay after being diluted with or dispersed or dissolved in water, physiological saline, buffer, etc. Needless to say, the endotoxin assay agent of the present invention also encompasses such diluted, dispersed, dissolved, or similarly processed form of the agent.

[0075]

In the endotoxin assay agent of the present invention, the factors and other components may be present as a mixture or present separately. For example, the factors may be mixed at any proportions in formulation, or individual factors may be formulated separately.

[0076]

The concentrations of the factors and other components contained in the endotoxin assay agent of the present invention are not particularly limited. However, each concentration is preferably adjusted so as to fall within the below-mentioned suitable concentration range for assaying

endotoxin. The concentration of each factor in the endotoxin assay agent of the present invention (as a solution prepared before contact with a test specimen) may be, for example, 5 to 200 $\mu\text{g/mL}$, and is preferably 20 to 100 $\mu\text{g/mL}$, more preferably 40 to 80 $\mu\text{g/mL}$, particularly preferably about 60 $\mu\text{g/mL}$. Also, the concentration of each factor in the endotoxin assay agent of the present invention may be adjusted in accordance with the method of producing the factor. When Factor C is produced with a rodent cell as a host, the concentration of Factor C in the endotoxin assay agent of the present invention (as a solution prepared before contact with a test specimen) can also be, for example, 5 to 200 $\mu\text{g/mL}$, 10 to 50 $\mu\text{g/mL}$, or 15 to 40 $\mu\text{g/mL}$. When Factor C is produced with a primate cell as a host, the concentration of Factor C in the endotoxin assay agent of the present invention (as a solution prepared before contact with a test specimen) can also be, for example, 5 to 200 $\mu\text{g/mL}$, 50 to 150 $\mu\text{g/mL}$, or 80 to 120 $\mu\text{g/mL}$. The above-exemplified concentrations may be calculated, for example, on the assumption that all the protein species present in the filtrate of a culture supernatant which has been obtained through expression of a factor of interest via the above-exemplified technique consists of the factor of interest.

[0077]

The endotoxin assay agent of the present invention may be provided as an endotoxin assay kit. The endotoxin assay kit is not particularly limited, so long as the kit comprises

the endotoxin assay agent of the present invention. The endotoxin assay kit may further comprise, for example, one or more elements selected from an endotoxin standard, a reaction container (e.g., a tube or a microplate), an instruction, and the like.

[0078]

(4) Endotoxin assay method of the present invention

When a test specimen contains endotoxin, the cascade reaction proceeds by mixing the endotoxin assay agent of the present invention with the test specimen. Through measuring the progress of the cascade reaction, endotoxin contained in the test specimen can be measured. That is, the present invention provides a method for measuring endotoxin present in a test specimen, the method comprising a step of mixing the endotoxin assay agent of the present invention and the test specimen, and a step of measuring the progress of a cascade reaction. The method is also referred to as the "endotoxin assay method of the present invention."

[0079]

In an embodiment of the endotoxin assay method of the present invention (hereinafter may be referred to as the "first embodiment"), all the factors are mixed with the test specimen. In the first embodiment, the factors contained the endotoxin assay agent of the present invention may be contained in the reaction system from the start of the step of mixing the endotoxin assay agent of the present invention with the test specimen, or may be successively added to the

reaction system.

[0080]

For example, the step of mixing the endotoxin assay agent of the present invention with the test specimen may comprise the following steps (A) to (C):

(A) a step of adding the Factor C of the present invention to the reaction system;

(B) a step of adding the Factor B of the present invention to the reaction system; and

(C) a step of adding the Pro-clotting enzyme to the reaction system.

[0081]

The steps (A) to (C) may be performed separately, partially simultaneously, or totally simultaneously. The steps (A) to (C) may be performed in any order. For example, the step (B) may be performed after the step (A), and the step (C) may be performed after the step (B).

[0082]

In the first embodiment, the progress of the cascade reaction can be measured by adding a detection substrate to the reaction system and measuring the response (coloring, coagulation, etc.) of the substrate. The detection substrate may be contained in the reaction system from the start of the step of mixing the endotoxin assay agent of the present invention with the test specimen, or may be added to the reaction system during the progress of the step or after completion of the step. Needless to say, the first

embodiment also encompasses an endotoxin assay method employing the endotoxin assay agent of the present invention which contains a detection substrate in advance.

[0083]

In the endotoxin assay method of the present invention, so long as the cascade reaction proceeds when the test specimen contains endotoxin, the Factor B and the Pro-clotting enzyme of the present invention *per se* are not need to be in contact with the test specimen. That is, another embodiment of the endotoxin assay method of the present invention (hereinafter may be referred to as a "second embodiment") is a method for measuring endotoxin present in a test specimen, the method comprising the following steps (A) to (D):

(A) a step of mixing the Factor C of the present invention with the test specimen;

(B) a step of mixing the Factor B of the present invention with the Factor C after mixing of step (A);

(C) a step of mixing the Pro-clotting enzyme of the present invention with the Factor B after mixing of step (B); and

(D) a step of measuring the progress of a cascade reaction.

[0084]

In the second embodiment, the steps (A) to (D) may be performed separately, partially simultaneously, or totally simultaneously. For example, step A may be initiated, and

then the Factor B and the Pro-clotting enzyme may be added to the reaction system during the progress of the step A or after completion of step A. Also, step B may be initiated, and then the Pro-clotting enzyme may be added to the reaction system during the progress of the step B or after completion of step B. Alternatively, all three factors may be incorporated into the reaction system from the start of step A. Still alternatively, the Factor C after the contact of step A may be recovered and used in step B, or the Factor B after the contact of step B may be recovered and used in step C.

[0085]

In the second embodiment, the progress of the cascade may be measured by adding a detection substrate to the reaction system and measuring the response (coloring, coagulation, etc.) of the substrate. The detection substrate may be contained in the reaction system from the start of step A, or may be added to the reaction system during the progress of any step or after completion of any step.

[0086]

Alternatively, endotoxin can be measured by detecting an intermediate stage of the cascade reaction. Specifically, endotoxin can be measured by detecting activated Factor C, which is an intermediate of the cascade reaction. That is, still another embodiment of the endotoxin assay method of the present invention (hereinafter may be referred to as a "third embodiment") may be a method for measuring endotoxin present

in a test specimen, the method comprising a step of mixing the Factor C of the present invention with the test specimen and a step of detecting activated Factor C. In the third embodiment, the activated Factor C can be detected by adding a detection substrate to the reaction system and measuring the response (coloring, etc.) of the substrate. The detection substrate may be contained in the reaction system from the start of the step of mixing the Factor C of the present invention with a test specimen, or may be added to the reaction system during the progress of the step or after completion of the step.

[0087]

The endotoxin assay method of the present invention, so long as the cascade reaction proceeds when the test specimen contains endotoxin, may further comprise any other arbitrary step. For example, the endotoxin assay method of the present invention may comprise a step of adding a detection substrate to the reaction system, or a step of mixing Clotting enzyme or an intermediate, which was yielded by the cascade reaction, with the detection substrate. Also, for example, the endotoxin assay method of the present invention may comprise a step of calculating the endotoxin level of the test specimen based on the reaction of the detection substrate.

[0088]

In the endotoxin assay method of the present invention, reaction is preferably carried out in an aqueous medium such

as water or a buffer.

[0089]

In the assay method of the present invention, the concentration of each factor in the reaction mixture is not particularly limited, so long as the cascade reaction proceeds when the test specimen contains endotoxin. The concentration of each factor may be appropriately tuned in accordance with properties of the factor and so forth. For example, the concentration of each factor, as a final concentration, may be, for example, 2.5 to 100 $\mu\text{g/mL}$, and is preferably 10 to 50 $\mu\text{g/mL}$, more preferably 20 to 40 $\mu\text{g/mL}$, particularly preferably about 30 $\mu\text{g/mL}$. Also, the concentration of each factor in the reaction mixture may be adjusted in accordance with the method of producing the factor. When Factor C is produced with a rodent cell as a host, the concentration of Factor C in the reaction mixture, as a final concentration, may also be, for example, 2.5 to 100 $\mu\text{g/mL}$, 5 to 25 $\mu\text{g/mL}$, or 7.5 to 20 $\mu\text{g/mL}$. When Factor C is produced with a primate cell as a host, the concentration of Factor C in the reaction mixture, as a final concentration, may also be, for example, 2.5 to 100 $\mu\text{g/mL}$, 25 to 75 $\mu\text{g/mL}$, or 40 to 60 $\mu\text{g/mL}$. The above-exemplified concentrations may be calculated, for example, on the assumption that all the protein species present in the filtrate of a culture supernatant which has been obtained through expression of a factor of interest via the above-exemplified technique consists of the factor of interest.

[0090]

In the assay method of the present invention, the concentration of the detection substrate in the reaction mixture is not particularly limited, so long as the cascade reaction proceeds when the test specimen contains endotoxin. The concentration of the detection substrate may be appropriately tuned in accordance with properties of the detection substrate and so forth. In the case where the detection substrate is a synthetic substrate, the concentration of the detection substrate is, for example, generally 0.001 mM to 100 mM, preferably 0.01 mM to 10 mM, as a final concentration.

[0091]

In any embodiment, so long as the cascade reaction proceeds when the test specimen contains endotoxin, the reaction system may further contain any other arbitrary component, in addition to the factors, the detection substrate, and the test specimen.

[0092]

The pH of the reaction mixture is not particularly limited, so long as the cascade reaction proceeds when the test specimen contains endotoxin. The pH of the reaction mixture may be appropriately tuned in accordance with properties of the factors. The pH of the reaction mixture is, for example, generally 5 to 10, preferably 7 to 8.5.

[0093]

The reaction temperature is not particularly limited,

so long as the cascade reaction proceeds when the test specimen contains endotoxin. The reaction temperature may be appropriately tuned in accordance with properties of the factors. The reaction temperature is, for example, generally 10°C to 80°C, preferably 20°C to 50°C. For example, the reaction temperature may be 37°C.

[0094]

The reaction time is not particularly limited. The reaction time may be appropriately tuned in accordance with various conditions such as properties of the factors and reaction temperature. The reaction time is, for example, generally 5 minutes to 2 hours, preferably 15 minutes to 90 minutes. The reaction time may be, for example, 30 minutes to 40 minutes.

[0095]

In any embodiment, the test specimen, the factors, and other components may be further added singly or in any combination to the reaction system during the course of reaction. These components may be added once or a plurality of times, or in a continuous manner. The reaction conditions may be constant from the start to the end of the reaction, or may be varied in the course of the reaction.

[0096]

The progress of the cascade reaction attributable to the presence of endotoxin can be measured by measuring the response (coloring, coagulation, etc.) of the detection substrate, whereby endotoxin in the test specimen can be

measured. The response (coloring, coagulation, etc.) of the detection substrate may be measured through a technique in accordance with the property of the detection substrate employed.

[0097]

In the case of quantitatively measuring endotoxin, correlation data between endotoxin level and the extent of response (coloring, coagulation, etc.) of the detection substrate are obtained by use of an endotoxin standard sample with known concentration, and then the amount of endotoxin present in the specimen can be quantitatively determined on the basis of the obtained correlation data. The correlation data is, for example, a calibration curve. The quantitation may be carried out through a kinetic method or an end-point method.

[0098]

The test specimen which can be subjected to endotoxin assay is not particularly limited. Examples of the specimen include water for medical use, pharmaceutical products, infusion solutions, blood products, medical apparatuses, medical instruments, cosmetics, foods and beverages, environmental specimens (e.g., air, river water, and soil), biological specimens (e.g., blood, body fluid, and tissue), native proteins, recombinant proteins, nucleic acids, and saccharides. The test specimen may be, for example, an injection. An injection refers to an agent that can be administered to the biological body via injection. Such an

injection is not necessarily fluid during commercial distribution, and may be a lyophilized product. The test specimen itself, or an extract or wash liquid thereof may be mixed, dispersed, or dissolved in the reaction system, to thereby be subjected to endotoxin assay.

[0099]

As described above, the Factor C of the present invention may have low susceptibility to inhibition of activity by an ion. Thus, in an embodiment of the present invention, endotoxin present in a test specimen containing an ion can be suitably measured.

[0100]

In other words, the test specimen of the invention may be a test specimen containing an ion. The expression "containing an ion" refers to the state that the test specimen contains a substance which is present in the form of an ion during endotoxin assay. That is, "ion" referred to in the present invention may be species which are present inherently in an ionized form in the test specimen, or may be species which are present inherently in a salt form in the test specimen but are ionized during endotoxin assay. The type of ions is not particularly limited. An example of the ion is a cation, and an example of the cation is a metal ion. Examples of the metal ion include an alkali metal ion and an alkaline earth metal ion. Specific examples of the alkali metal ion and the alkaline earth metal ion include sodium ion, potassium ion, calcium ion, and magnesium ion. The

contained amount of the ion in the test specimen may be such an amount that, for example, the concentration of the cation derived from the test specimen becomes 1 mM or higher, 2 mM or higher, 5 mM or higher, 10 mM or higher, 20 mM or higher, 50 mM or higher, or 100 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the endotoxin assay agent. Specific examples of the salt include sodium chloride, magnesium sulfate, sodium hydrogencarbonate, sodium citrate, calcium chloride, potassium chloride, sodium iothalamate, calcium disodium edetate, and dihydrogen sodium phosphate. The contained amount of the salt in the test specimen may be such an amount that, for example, the concentration of the salt derived from the test specimen becomes 1 mM or higher, 2 mM or higher, 5 mM or higher, 10 mM or higher, 20 mM or higher, 50 mM or higher, or 100 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the endotoxin assay agent. The test specimen may contain one kind of ion (or salt) or two or more kinds of ions (or salts).

Examples

[0101]

The present invention will next be described more particularly by way of examples. However, these are merely examples of the present invention, and the scope of the present invention is not limited to these.

[0102]

1. Preparation of Factor C proteins

(1) Expression of Factor C in insect cells (Sf9)

In this section, Factor C was prepared by use of insect cell Sf9 as a host in the following manner.

[0103]

A DNA encoding Factor C of Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *EcoRV* recognition site and the *MluI* recognition site of pIZ-V5 (product of Life Technologies Corporation), which is a vector for expression in insect cells, to thereby construct a Factor C expression plasmid. The nucleotide sequence of the DNA is shown in SEQ ID NO: 1, and the amino acid sequence of Factor C encoded by the DNA is shown in SEQ ID NO: 2 (the same applies throughout the Example).

[0104]

The Factor C expression plasmid was introduced into cultured Sf9 cells through transfection by use of Celfectin reagent (product of Life Technologies Corporation). The Sf9 cells were statically cultured at 28°C in an Sf900 III serum-free medium (product of Life Technologies Corporation) containing 300 to 600 µg/mL Zeocin (product of Life Technologies Corporation), whereby Sf9 strains into which the genome the expression plasmid had been incorporated were selected.

[0105]

Candidates for Sf9 in which the Factor C gene had been

stably expressed were isolated through the cloning cylinder technique, and a plurality of Sf9 cell lines was cloned. The thus-obtained clones were individually cultured, to thereby obtain culture supernatants. Secretion of the recombinant Factor C protein in each culture supernatant was confirmed through western blotting by use of an anti-Factor C antibody (2C12 antibody; Yoshiki Miura, *et al.*, J. Biochem. 112: 476-481 (1992)). Also, activity of Factor C (property of being activated by endotoxin) in the culture supernatant was confirmed through activity measurement by use of a synthetic substrate Boc-LGR-pNA. Notably, the procedure of activity measurement is described in "4. Activity measurement" below (the same applies throughout the Example). Based on the results of western blotting and activity measurement, an Sf9 cell line highly expressing Factor C was selected.

[0106]

The Sf9 cell line highly expressing Factor C was suspension-cultured (28°C) in an Sf900 III medium containing 1× penicillin/streptomycin (product of Life Technologies Corporation) and 50 µg/mL Zeocin to a later phase of the logarithmic growth phase (6 to 8×10⁶ cells/mL). Then, the culture was centrifuged (3,000×g, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22-µm filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (Sf9).

[0107]

(2) Expression of Factor C in mammalian cells (CHO DG44)

In this section, Factor C was prepared by use of CHO DG44, which is a Chinese hamster ovary cell line, as a host through the following procedure.

[0108]

A DNA encoding Factor C of Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *EcoRI* recognition site and the *XbaI* recognition site of pCI-neo (product of Promega), which is a vector for expression in mammalian cells, to thereby construct a Factor C expression plasmid. A kozak sequence (GCCACC) was added just before the start codon of Factor C.

[0109]

The Factor C expression plasmid and a dhfr expression plasmid were incorporated into cultured CHO DG44 cells through simultaneous transfection using a lipofection reagent (Lipofectamine LTX) (product of Life Technologies Corporation). The CHO DG44 cells were statically cultured at 37°C under feeding of 5% CO₂ in an RPMI 1640 medium (5% dialyzed serum, containing 1× penicillin/streptomycin) (product of Sigma) added with 1 mg/mL Geneticin (product of Life Technologies Corporation), whereby CHO DG44 strains into which the genome the expression plasmids had been incorporated were selected.

[0110]

Fifteen days after the simultaneous transfection, MTX (methotrexate) (product of Wako Pure Chemical Industries,

Ltd.) was added to the culture medium. The MTX concentration was elevated stepwise from 50 nM to 5,000 nM over three months, and CHO DG44 cell lines highly expressing Factor C were selected as polyclones. Effective amplification of the incorporated Factor C gene by virtue of MTX was confirmed by detecting Factor C in a culture supernatant through western blotting using a 2C12 antibody, and quantitating the transcriptional amount of mRNA of Factor C through real-time PCR.

[0111]

The group of polyclonal CHO DG44 cell lines highly expressing Factor C was diluted by the medium to obtain a cell concentration of 0.5 cells/well, and the dilute was inoculated to a 96-well plate. From about 10 to 20 days after inoculation, the formed cell colonies were successively subjected to expansion culture, whereby CHO DG44 cell lines highly expressing Factor C were cloned. The transcriptional amount of mRNA of Factor C was confirmed through real-time PCR. The amount of Factor C protein secreted to the medium was confirmed through western blotting using a 2C12 antibody. The Factor C activity (property of being activated by endotoxin) in the culture supernatant was confirmed through activity measurement using a synthetic substrate Boc-LGR-pNA. Based on these results, a monoclonal CHO DG44 cell line highly expressing Factor C was selected.

[0112]

The CHO DG44 cell line highly expressing Factor C

(monoclonal) was conditioned in a serum-free complete synthetic medium containing 5 μ M MTX and thus floated. The cell line was grown at 37°C under feeding of 5% CO₂ to a later phase of the logarithmic growth phase. The thus-obtained floating culture broth was centrifuged (3,000×g, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22- μ m filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (CHO).

[0113]

(3) Expression of Factor C in mammalian cells (HEK293)

In this section, Factor C was prepared by use of HEK293, which is a human embryo kidney cell line, as a host through the following procedure.

[0114]

A DNA encoding Factor C of a Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *Eco*RI recognition site and the *Xho*I recognition site of pCA7 (Makoto Takeda, *et al.*, J. Virol. 79 (22): 14346-54 (2005)), which is a vector for expression in mammalian cells, to thereby construct a Factor C expression plasmid. A kozak sequence (GCCACC) was added just before the start codon of Factor C.

[0115]

The Factor C expression plasmid was incorporated into cultured HEK293 cells through transfection using polyethylenimine (product of Sigma) (Hashiguti *et al.*,

Expression of Recombinant Protein Using Cultured Human Cells
-Standard Protocol by 293-type cells-, PSSJ Archives, 1, e017
(2008); available on the world wide web at
pssj.jp/archives/Protocol/Expression/293_01/293_01_01.html.
HEK293 cells were statically cultured in a DMEM medium
(containing 10% fetal bovine serum and 2mM L-glutamine)
(product of Sigma) at 37°C under feeding of 5% CO₂ without
replacement of the medium. Four days after transfection, the
culture broth was recovered, and was centrifuged (3,300×g, 30
minutes, 4°C), to thereby recover a culture supernatant. The
supernatant was filtered through a 0.22-μm filter, and the
thus-recovered fraction (i.e. filtrate) was employed as
recombinant Factor C (HEK).

[0116]

(4) Preparation of native Factor C (derived from amebocyte of
Tachypleus tridentatus)

A fraction containing Factor B and Factor C was
recovered from an amebocyte extract of Japanese horseshoe
crab, *Tachypleus tridentatus*, through column chromatography
by use of dextran sulfate resin (Takanori Nakamura, *et al.*,
Eur. J. Biochem. 154: 511-521 (1986)). The fraction (5 mL)
was subjected to gel filtration by means of a column (ϕ 2.2
cm × 97 cm) filled with Sepharose CL6B gel (product of GE
Healthcare). Gel filtration was performed under almost the
same conditions as employed in a known technique (Jeak Ling
Ding and Bow Ho, US 5,712,144; Jeak L. Ding, *et al.*, Biochem.
Biophys. Acta 1202: 149-156). The flow rate was adjusted to

15 mL/h, and 50 mM Tris buffer (pH 8.0) containing 154 mM NaCl, 1 mM EDTA, and 5% DMSO was employed as a solvent.

[0117]

Gel filtration eluted fractions (3.7 mL/tube, 150 tubes) were subjected to SDS-PAGE followed by CBB staining and western blotting using a 2C12 antibody, and activity measurement using a synthetic substrate Boc-LGR-pNA, whereby fractions containing Factor C protein were identified. These fractions containing the purified Factor C protein were employed as native Factor C (TAL).

[0118]

2. Preparation of recombinant Factor B and recombinant Pro-clotting enzyme

A DNA encoding Factor B of Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *EcoRV* recognition site and the *MluI* recognition site of pIZ-V5 (product of Life Technologies Corporation), which is a vector for expression in insect cells, to thereby construct a Factor B expression plasmid. The nucleotide sequence of the DNA is shown in SEQ ID NO: 9, and the amino acid sequence of Factor B encoded by the DNA is shown in SEQ ID NO: 6. Notably, the nucleotide sequence shown in SEQ ID NO: 9 is a nucleotide sequence optimized for expression in insect cells.

[0119]

A DNA encoding Pro-clotting enzyme of Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *EcoRV* recognition site and the *MluI* recognition site of

pIZ-V5 (product of Life Technologies Corporation), which is a vector for expression in insect cells, to thereby construct a Pro-clotting enzyme expression plasmid. The nucleotide sequence of the DNA is shown in SEQ ID NO: 7, and the amino acid sequence of Pro-clotting enzyme encoded by the DNA is shown in SEQ ID NO: 8.

[0120]

Through the same method as employed in the case of Factor C, the expression plasmids were each incorporated into Sf9 cells, high-expression clones were selected, and culture supernatants thereof were prepared. The culture supernatants were individually filtered through a 0.22- μ m filter, and the obtained fractions (i.e. filtrates) were employed as a recombinant Factor B and a recombinant Pro-clotting enzyme. Recovery of the factors was confirmed through western blotting employing an antibody specific to each factor and activity measurement.

[0121]

3. Comparison of molecular weights of Factor C proteins (1)

The molecular weights of the above-prepared four types of Factor C proteins were compared with one another through SDS-PAGE and western blotting.

[0122]

Firstly, each Factor C in an amount shown in Fig. 2 was subjected to SDS-PAGE under a non-reducing condition employing a 5 to 20% gradient gel. In Fig. 2, Sf9, CHO, and HEK denote the Factor C expressed in Sf9 cells, CHO DG44

cells, and HEK293 cells, respectively, and TAL refers to native Factor C (hereinafter the same applies). Notably, Factor C is cleaved into an H chain and an L chain under reducing conditions. In the present Example, SDS-PAGE was carried out under a non-reducing condition in order to compare the molecular weights of Factor C in an intact state.

[0123]

After SDS-PAGE, proteins were transferred to a PVDF membrane (product of Bio RAD) by means of a semi-dry blotter. The PVDF membrane was recovered and blocked with 5% skim milk, and then sequentially treated with a primary antibody (2C12 antibody) and a secondary antibody (HRP-labeled anti-mouse goat antibody) (product of Dako). Bands attributed to Factor C were made to emit light with a detection reagent, SuperSignal West Dura (product of Thermo Scientific), and the light emission was recorded by a CCD camera.

[0124]

The four types of Factor C were found to have different molecular weights, and the molecular weight was the greatest in the case of CHO, followed by TAL, HEK, and Sf9 in the descending order (Fig. 2).

[0125]

Regarding Singaporean horseshoe crab, *Carcinoscorpius rotundicauda*, it has been reported that all of the native Factor C protein purified from an amebocyte extract, and recombinant Factor C proteins produced in insect cells Sf9 and S2, and in African green monkey kidney cell line COS-1 as

a host cell have the same molecular weight of 132 kDa (Jeak Ling Ding and Bow Ho, US 5,712,144, and Jeak L. Ding, et al., Biochem. Biophys. Acta 1202: 149-156 (1993) (for native Factor C protein); Jing Wang, et al., J. Biol. Chem. 277(39): 36363-72 (2002) (for Sf9 and S2); and Roopashree S. Dwarakanath, et al., Biotechnology letters 19(4): 357-361 (1997) (for COS-1)).

[0126]

Thus, among the above-prepared four types of Factor C, the molecular weight of each of CHO and TAL was correctly measured. Firstly, recombinant Factor C (CHO) was purified in a manner similar to that employed in the case of native Factor C (TAL). Then, electrophoresis (15% gel, LMW Marker kit (product of GE Healthcare)) was performed under the same conditions as employed in a known technique (Jeak Ling Ding and Bow Ho, US 5,712,144; and Jeak L. Ding, et al., Biochem. Biophys. Acta 1202: 149-156), and the molecular weight of each Factor C was then determined through Ferguson plotting. As a result, recombinant Factor C (CHO) was found to have a molecular weight of 126 kDa, and native Factor C (TAL) was found to have a molecular weight of 123 kDa (Fig. 3). Thus, native Factor C (TAL) and recombinant Factor C (CHO) derived from Japanese horseshoe crab, *Tachypleus tridentatus*, were found to have a molecular weight smaller than that of the Factor C derived from Singaporean horseshoe crab, *Carcinoscorpius rotundicauda* (132 kDa).

[0127]

The difference in molecular weight among the above-prepared four types of Factor C is conceived to be attributed to the difference in post-translation modification (sugar chain modification) depending on the type of host cells employed. For example, it has been known that sialic acid is linked to the N-type sugar chain after translation in mammalian cells, whereby a recombinant protein produced in mammalian cells has dimensions larger than those of a recombinant protein produced in insect cells (Robert L. Harrison and Donald L. Jarvis, *Methods in Molecular Biology* 338: 341-356 (2007)). This supports the results of the present Example (Fig. 2).

[0128]

4. Activity measurement

Each of the above-prepared four types of Factor C was combined with other factors, and activity measurement was carried out through the following procedure. Fig. 4 shows the scheme of activity measurement.

[0129]

As shown in Fig. 4, common materials other than Factor C (a recombinant Factor B, a recombinant Pro-clotting enzyme, a synthetic substrate Boc-LGR-pNA, and Tris buffer) were mixed together, to thereby prepare a common liquid mixture. To the common liquid mixture, Factor C in an amount specified in Fig. 6 was added, and the total volume was adjusted to 50 μ L. The thus-obtained mixture was mixed with 50 μ L of a specimen (water containing United States Pharmacopoeia

standard endotoxin (USP-RSE) (product of Seikagaku corporation)), and the resultant mixture was heated at 37°C for 30 minutes. In this assay system, Factor C is activated by endotoxin, to thereby form activated Factor C; Factor B is activated by the activated Factor C, to thereby form activated Factor B; Pro-clotting enzyme was activated by the activated Factor B, to thereby form a corresponding Clotting enzyme; and the Clotting enzyme cleaves a synthetic substrate Boc-LGR-pNA, whereby p-nitroaniline is released (Fig. 5). The change in absorbance (A 405 nm) attributed to formation of p-nitroaniline was measured in a time-dependent manner, and the absorbance change rate per unit time (mAbs/min) was calculated and employed as Factor C activity. The Measurement was conducted thrice for each sample.

[0130]

In the above assay system, the final concentration of each factor was as follows: recombinant Factor B 30 µg/mL, recombinant Pro-clotting enzyme 30 µg/mL, recombinant Factor C (Sf9) 11.3 µg/mL, recombinant Factor C (CHO) 12 µg/mL, recombinant Factor C (HEK) 51.5 µg/mL, and Factor C (TAL) 0.25 µg/mL. The above-exemplified concentrations (except for the case of TAL) were calculated, on the assumption that all the protein species present in the sample (i.e. filtrate) prepared through expression of a factor of interest consisted of the factor of interest. The Factor C (TAL) concentration was measured after purification of Factor C (TAL).

[0131]

As a result, reconstituted systems each exhibiting almost equivalent activity were successfully constituted for the four types of Factor C (Fig. 6). In each reconstituted system, the activity was increased with an increase in endotoxin concentration (0.05 and 0.1 EU/mL) of a specimen.

[0132]

The recombinant Factor C derived from *Carcinoscorpius rotundicauda* and produced in mammalian cell COS-1 (132 kDa) is reported to be recovered in an insoluble fraction (Roopashree S. Dwarakanath, et al., Biotechnology letters 19(4): 357-361 (1997); and Jing Wang, Bow Ho and Jeak L. Ding, Biotechnology letters 23: 71-76 (2001)). Also, the recombinant Factor C derived from *Carcinoscorpius rotundicauda* and produced in insect cell S2 (132 kDa) is reported to exhibit no protease activity in response to endotoxin (Jing Wang, et al., J. Biol. Chem. 277(39): 36363-72 (2002)). Lack of activity of the latter Factor C (produced in S2) is thought to be attributed to the difference in sugar chain structure from the native Factor C derived from *Carcinoscorpius rotundicauda* and the recombinant Factor C produced in insect cell Sf9, due to difference in reactivity to lectin.

[0133]

Meanwhile, in the present Example, it was found that the recombinant Factor C derived from *Tachyplesus tridentatus* (Sf9, CHO, and HEK) exhibited an endotoxin-dependent activity, even though the molecular weight thereof differs

from that of native Factor C (TAL); i.e., even though the sugar chain structure thereof differs from that of native Factor C (TAL).

[0134]

5. Inhibition of activity by injection

Endotoxin is a strong immune reaction-inducing substance. Thus, when an injection contaminated with endotoxin is administered to the body, a grave side effect occurs. Therefore, in production of injections, detection of endotoxin by use of a limulus amebocyte lysate reagent is obligated by the pharmacopoeia of the relevant country, to thereby confirm no endotoxin contamination.

[0135]

Thus, the above-prepared four types of Factor C were compared in terms of reactivity to endotoxin in various injections.

[0136]

As a control specimen, injection water containing endotoxin (endotoxin concentration: 0.05 EU/mL) was used. Measurement specimens used were as follows: 4-fold dilute of 10 w/v% sodium chloride injection (10% sodium chloride injection, product of Otsuka Pharmaceutical Co., Ltd.); 16-fold dilute of 0.5 M magnesium sulfate injection (Mg sulfate corrective injection 1 mEq/mL, product of Otsuka Pharmaceutical Co. Ltd.); undiluted solution of physiological saline (Otsuka isotonic sodium chloride solution, product of Otsuka Pharmaceutical Co., Ltd.); 8-fold dilute of 7 w/v%

sodium hydrogencarbonate injection (Meylon injection 7%, product of Otsuka Pharmaceutical Co., Ltd.); 8-fold dilute of 10 w/v% sodium citrate injection (sodium citrate injection for transfusion, product of Fuso Pharmaceutical Industries, Ltd.); 100-fold dilute of 0.5 M calcium chloride injection (Ca chloride corrective injection 1 mEq/mL, product of Otsuka Pharmaceutical Co., Ltd.); undiluted solution of Ringer's solution (Ringer's solution "Fuso", product of Fuso Pharmaceutical Industries, Ltd.); and 16-fold dilute of sodium iothalamate injection (Conray 400 injection, product of Daiichi Sankyo Co., Ltd.), each spiked with endotoxin (endotoxin concentration: 0.05 EU/mL). When the measurement specimen was 8-fold dilute of 10 w/v% sodium citrate injection, the final sodium citrate concentration was about 21 mM. When the measurement specimen was 8-fold dilute of 7 w/v% sodium hydrogencarbonate injection, the final sodium hydrogencarbonate concentration was about 52 mM. When the measurement specimen was 4-fold dilute of 10 w/v% sodium chloride injection, the final sodium chloride concentration was about 214 mM. When the measurement specimen was undiluted solution of physiological saline, the final sodium chloride concentration was about 77 mM. When the measurement specimen was 16-fold dilute of 0.5 M magnesium sulfate injection, the final magnesium sulfate concentration was about 16 mM. When the measurement specimen was 100-fold dilute of 0.5 M calcium chloride injection, the final calcium chloride concentration was about 2.5 mM. Activity of Factor

C was measured through the procedure shown in Fig. 4. A residual activity was defined as a relative reactivity (%) for endotoxin in an injection specimen, with respect to the reactivity for endotoxin in the corresponding control specimen as 100%. The measurement was carried out four times for each injection specimen.

[0137]

Fig. 7 shows the results. When a typical injection was employed as a measurement specimen, reaction with endotoxin was inhibited, as compared with the case in which injection water was used. The degree of inhibition varied in accordance with the type of injection and the type of host for expressing Factor C. Recombinant Factor C (CHO) and recombinant Factor C (HEK) showed a tendency to have low susceptibility to reaction inhibition to all the tested injections, as compared with native Factor C (TAL). Particularly, recombinant Factor C (CHO) exhibited the highest residual activity in many cases. In contrast, recombinant Factor C (Sf9) was most susceptible to reaction inhibition in many injections (7 injections except sample No. 6).

[0138]

The test results indicate that Factor C expressed in mammalian cells can have low susceptibility to activity inhibition by ions. Such a property is particularly advantageous for detection of endotoxin contamination in production of injections.

[0139]

6. Preparation of L chain-specific Factor C protein antibody

A Factor C protein is known to be present as a single chain (Full chain) under non-reducing conditions, and to be cleaved into two chains (H chain and L chain) under reducing conditions. The anti-Factor C monoclonal antibody (2C12 antibody) is reported to recognize the Full chain and the H chain (Yoshiki Miura, *et al.*, J. Biochem. 112: 476-481(1992)). Thus, a peptide antigen present in the L chain was produced. A rabbit was immunized with the peptide antigen through a conventional method, whereby a polyclonal antibody which can recognize the L chain and the Full chain (FCL antibody) was produced (Fig. 10).

[0140]

7. Purification of recombinant Factor C proteins

According to the method employed in "(4) Preparation of native Factor C (derived from *Tachypleus tridentatus*)" of "1. Preparation of Factor C proteins", three types of recombinant Factor C proteins were purified from culture supernatants of mammalian cells (CHO DG44, and HEK293) and insect cells (Sf9). Briefly, each supernatant (100 to 300 mL) was subjected to column chromatography by use of dextran sulfate resin, and elution fractions containing the corresponding recombinant Factor C were isolated through gel filtration. Purification of the recombinant Factor C proteins was confirmed through SDS-PAGE and CBB staining, western blotting employing the 2C12 antibody, and activity measurement.

[0141]

8. Comparison of molecular weights of Factor C proteins (2)

The molecular weights of the above-purified four types of Factor C proteins were compared again with one another through SDS-PAGE and western blotting.

[0142]

The four types of purified Factor C proteins, derived from a native horseshoe crab (TAL), a culture supernatant of CHO DG44 (CHO), a culture supernatant of HEK293 (HEK), and a culture supernatant of Sf9 (Sf9), were applied to a single gel (15% gel) for SDS-PAGE, and subjected to CBB staining (Fig. 11). Each of the four types of purified Factor C proteins presented as a single chain (Full chain) under non-reducing conditions, and was cleaved into two chains (H chain and L chain) under reducing conditions. After transfer to the PVDF membrane, western blotting was performed by use of the 2C12 antibody and the FCL antibody (Fig. 12). In relation to antibody recognition sites (Fig. 10), the 2C12 antibody specifically recognized the Full chain and the H chain, and the FCL antibody specifically recognized the Full chain and the L chain. The CBB-stained bands shown in Fig. 11 were identified to bands derived from Factor C. Regarding molecular weight of Factor C, CHO had the largest molecular weight; TAL and HEK had almost the same molecular weight; and Sf9 has the smallest molecular weight, in all cases of the Full chain under non-reducing conditions and of the H-chain and L chain under reducing conditions.

[0143]

In order to more precisely compare the molecular weights of the four types of purified Factor C, electrophoresis (15% gel, LMW marker (product of GE Healthcare)) was performed under the same conditions as employed in a known technique (Jeak Ling Ding and Bow Ho, US 5,712,144; and Jeak L. Ding, *et al.*, Biochem. Biophys. Acta 1202: 149-156), followed by CBB staining, and the molecular weights were then determined through Ferguson plotting.

[0144]

Table 1 shows the results. Under non-reducing conditions, TAL had a molecular weight of 124 kDa, which is almost equivalent to 123 kDa reported by the reference. The molecular weight of CHO was higher, 128 kDa, and that of HEK was 124 kDa, which is equivalent to that of TAL. The molecular weight of Sf9 was 109 kDa, which is smaller than that of TAL. As a result, when recombinant Factor C was expressed in insect cells (Sf9), the molecular weight thereof was found to be smaller than that of native Factor C (TAL), whereas when recombinant Factor C was expressed in mammalian cells (CHO, HEK), the molecular weight thereof was found to be equivalent to or larger than that of native Factor C (TAL). This tendency was also confirmed in the case of the H chain or the L chain under reducing conditions.

[0145]

The molecular weight of native Factor C derived from *Carcinoscorpius rotundicauda* (Singaporean horseshoe crab) and

that of recombinant Factor C thereof obtained via gene expression in insect cells have been reported to be the same value, 132 kDa, under non-reducing conditions. The four types of purified Factor C derived from *Tachyplesus tridentatus* (Japanese horseshoe crab) and obtained in the present Example were found to have a molecular weight smaller than 132 kDa (Table 1).

[0146]

[Table 1]

Species of horseshoe crab	Source	Mol. wt. (kDa)			Reference
		Full	H	L	
<i>Tachypleus tridentatus</i>	Natural lysate	124	85	42	Present Example
<i>Tachypleus tridentatus</i>	CHO DG44	128	88	43	Present Example
<i>Tachypleus tridentatus</i>	HEK293	124	85	42	Present Example
<i>Tachypleus tridentatus</i>	Sf9	109	75	39	Present Example
<i>Tachypleus tridentatus</i>	Natural lysate	123	80	43	Takanori Nakamura, <i>et al.</i> , Eur. J. Biochem. 154: 511-521 (1986)
<i>Carcinoscorpius rotundicauda</i>	Natural lysate	132	80	52	Jeak L. Ding, <i>et al.</i> , Biochem. Biophys. Acta 1202: 149-156 (1993)
<i>Carcinoscorpius rotundicauda</i>	Sf9 (+baculo virus)	132	No data	No data	Jing Wang, <i>et al.</i> , J. Biol. Chem. 277 (39): 36363-72 (2002)
<i>Carcinoscorpius rotundicauda</i>	S2	132	No data	No data	Jing Wang, <i>et al.</i> , J. Biol. Chem. 277 (39): 36363-72 (2002)

[0147]

9. Confirmation of N-type sugar chain modification

The genes employed in the present Example for expressing the recombinant Factor C were sequences all derived from *Tachypleus tridentatus*, and the expression plasmids were designed so that no tag was added to any terminus of each sequence. Therefore, the translated amino acid sequences of the four types of Factor C obtained in the present Example are thought to be the same one. In addition, the partial sequences of the N-terminal amino acid residues

of the recombinant Factor C (HEK, Sf9) were determined. These amino acid sequences are identical to the sequence of the native Factor C of *Tachypleus tridentatus*, reported by the reference (data not shown).

[0148]

Generally, the molecular weight of protein is thought to vary considerably by modification after translation. In addition, it has also been reported that the structure of an N-type sugar chain added to protein through modification after translation differs between the case of insect cells and the case of mammalian cells. Thus, in order to confirm whether the above-observed difference in molecular weight among the purified Factor C proteins is attributed to the difference in N-type sugar chain structure, the Factor C proteins each were treated with glycopeptidase F. This enzyme is known to cleave and remove an N-type sugar chain added to protein. Therefore, when the purified Factor C proteins have the same molecular weight after treatment with the enzyme, N-type sugar chain is conceived to induce the difference in molecular weight among the Factor C proteins.

[0149]

According to an attached instruction, each purified Factor C protein (1 μ g) was treated with 1 mU glycopeptidase F (product of Takara) under modification conditions in the presence of SDS and a reducing agent. The thus-treated samples were separated through SDS-PAGE, and then analyzed through western blotting. Fig. 13 shows the results. In all

cases of the four types of Factor C, the molecular weight of H chain decreased by the glycopeptidase treatment and became the same value. By contrast, although the molecular weight of L chain decreased after the glycopeptidase treatment in all cases of the four types of Factor C, the molecular weight became the same value only in the cases of CHO, HEK, and TAL. Specifically, among four types of L chains, the L chain of the Factor C derived from Sf9 was found to have a molecular weight larger than that of the other three cases, after the glycopeptidase treatment. Therefore, in the L chain of the Factor C derived from Sf9, a certain modification other than N-type sugar chain modification was conceived to occur, after translation.

[0150]

Thus, the aforementioned analysis indicates that the difference in mode of N-type sugar chain after translation plays a main role in providing the difference in molecular weight of Factor C.

[0151]

10. Analysis of N-type sugar chain of Factor C

In N-type sugar chain modification of a protein in mammalian cells, sugar molecules are stepwise added to an asparagine residue of the protein, and eventually, a sugar chain containing terminal sialic acid (i.e., complex-type) is formed. In contrast, in insect cells, sialic acid is not generally added, and a sugar chain having a mannose terminus (i.e., paucimannose-type) is formed (Robert L. Harrison and

Donald L. Jarvis, *Methods in Molecular Biology* 338: 341-356 (2007)). In order to confirm the difference in N-type sugar chain structure among the four types of purified Factor C proteins, lectin blotting was performed by use of various lectins such as terminal sialic acid-specific lectins (e.g., *Maackia amurensis* agglutinin (MAM) and *Sambucus sieboldiana* agglutinin (SSA)).

[0152]

Dilution series (100, 25, and 6 ng) samples of each of the four types of purified Factor C and bovine fetuin protein as a positive control were dot-blotted onto a nitrocellulose membrane, and the membrane was blocked with 3% BSA. Then, the membrane was sequentially reacted with biotin-labeled lectin (product of J-Oil Mills, Inc.) and streptavidin-labeled HRP (product of Thermo Scientific). Light emission attributed to decomposition of the HRP substrate (product of Thermo Scientific) was detected. Instead of lectin, a 2C12 anti-Factor C antibody was used as a control, to thereby confirm that the same amounts of four types of Factor C were blotted.

[0153]

Fig. 14(a) shows the results of lectin blotting, and Fig. 14(b) shows the binding specificity of lectins employed. *Lens culinaris* agglutinin (LCA) (fucose-specific lectin) and *Canavalia ensiformis* agglutinin (Con A) (bi-antenna-type, high-mannose-type, and complex-type sugar chain-specific lectin) were bonded to all the samples; i.e., four types of

Factor C and fetuin. In contrast, MAM ($(\alpha-2,3)$ -linked terminal sialic acid-specific lectin) was strongly bonded to CHO and less strongly bonded to HEK. TAL and fetuin have almost the same weak reactivity, but did not react with Sf9. SSA ($(\alpha-2,6)$ -linked terminal sialic acid-specific lectin) reacted with HEK, TAL, and fetuin, but did not react with CHO and Sf9.

[0154]

The above analysis indicates that the N-type sugar chain of recombinant Factor C expressed in mammalian cells (CHO and HEK) contains $(\alpha-2,3)$ -linked terminal sialic acid, and that the N-type sugar chain of recombinant Factor C expressed in insect cells (Sf9) contains no $(\alpha-2,3)$ -linked terminal sialic acid. Fig. 14(c) shows schematic structures of the N-type sugar chain of recombinant Factor C, estimated by the results of the above experiments and reports by the reference. The results of the above experiments support that the difference in mode of N-type sugar chain modification after translation is mainly attributed to the difference in molecular weight of Factor C.

Industrial Applicability

[0155]

According to the present invention, a recombinant horseshoe crab Factor C can be efficiently produced. In an embodiment of the present invention, endotoxin present in a test specimen containing an ion can be measured, while

inhibition of reaction is reduced.

[0156]

Sequence listing

SEQ ID NO: 1: DNA sequence of the Factor C gene of Japanese horseshoe crab

SEQ ID NO: 2: Amino acid sequence of the Factor C of Japanese horseshoe crab

SEQ ID NO: 3: DNA sequence of the Factor C gene of Southeast Asian (Singaporean) horseshoe crab

SEQ ID NO: 4: Amino acid sequence of the Factor C of Southeast Asian (Singaporean) horseshoe crab

SEQ ID NO: 5: DNA sequence of the Factor B gene of Japanese horseshoe crab

SEQ ID NO: 6: Amino acid sequence of the Factor B of Japanese horseshoe crab

SEQ ID NO: 7: DNA sequence of the Pro-clotting enzyme gene of Japanese horseshoe crab

SEQ ID NO: 8: Amino acid sequence of the Pro-clotting enzyme of Japanese horseshoe crab

SEQ ID NO: 9: DNA sequence of a Factor B gene of Japanese horseshoe crab, the gene having a codon optimized for expression in insect cells

SEQ ID NO: 10: Peptide sequence

What is claimed is:

1. A method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor C having activity of Factor C,

expressing the horseshoe crab Factor B in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor B having activity of Factor B,

expressing the horseshoe crab Pro-clotting enzyme in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme, and

formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.

2. The method according to claim 1, wherein the mammalian cell expressing the Factor B is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

3. The method according to claim 1, wherein the mammalian

cell expressing the Pro-clotting enzyme is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.

4. The method according to claim 1, wherein the horseshoe crab is selected from the group consisting of *Tachyplesus tridentatus*, and *Limulus polyphemus*.

5. The method according to claim 1, wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

6. The method according to claim 1, wherein the horseshoe crab Factor C has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

7. The method according to claim 1, further comprising filtering the culture supernatant comprising the horseshoe crab Factor C.

8. The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are mixed in a formulation.

9. The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are formulated separately.

10. A method for measuring endotoxin in a test specimen, the method comprising a step of mixing an endotoxin assay agent produced by the method according to claim 1 and a test specimen, and a step of measuring a progress of a cascade reaction.

Abstract

A horseshoe crab Factor C protein having activity of Factor C, wherein the horseshoe crab is selected from *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell or HEK cell.

UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>		Attorney Docket No.	Q289014
		First Named Inventor	Hikaru MIZUMURA
		Title	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN
		Priority Mail Express® Label No.	
APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>		Commissioner for Patents ADDRESS TO: P.O. Box 1450 Alexandria VA 22313-1450	
1. ☒ Fee Transmittal Form (PTO/SB/17 or equivalent) 2. ☐ Applicant asserts small entity status. See 37 CFR 1.27 3. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent. 4. ☒ Specification [Total Pages <u>104</u>] Both the claims and abstract must start on a new page. <i>(See MPEP § 608.01(a) for information on the preferred arrangement)</i> 5. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets <u>10</u>] 6. Inventor's Oath or Declaration [Total Pages <u>3</u>] <i>(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))</i> a. ☐ Newly executed (original or copy) b. ☒ A copy from a prior application (37 CFR 1.63(d)) 7. ☒ Application Data Sheet. *See note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent) 8. CD-ROM or CD-R in duplicate, large table or Computer Program (<i>Appendix</i>) ☐ Landscape Table on CD 9. ☒ Nucleotide and/or Amino Acid Sequence Submission (ST.26 Sequence Listing (xml))		ACCOMPANYING APPLICATION PAPERS 10. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee <u>SEIKAGAKU CORPORATION</u> 11. ☐ 37 CFR 3.73(c) Statement ☒ Power of Attorney <i>(when there is an assignee)</i> 12. ☐ English Translation Document <i>(if applicable)</i> 13. ☒ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☐ Copies of citations attached 14. ☒ Preliminary Amendment 15. ☐ Return Receipt Postcard <i>(MPEP § 503)(Should be specifically itemized)</i> 16. ☐ Certified Copy of Priority Document(s) <i>(if foreign priority is claimed)</i> 17. ☐ Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent. 18. ☒ Other: Continuation of U.S. Application No. 17/554,128; Track One Request	
<p>*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 must be included in an Application Data Sheet (ADS). (2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).</p>			
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