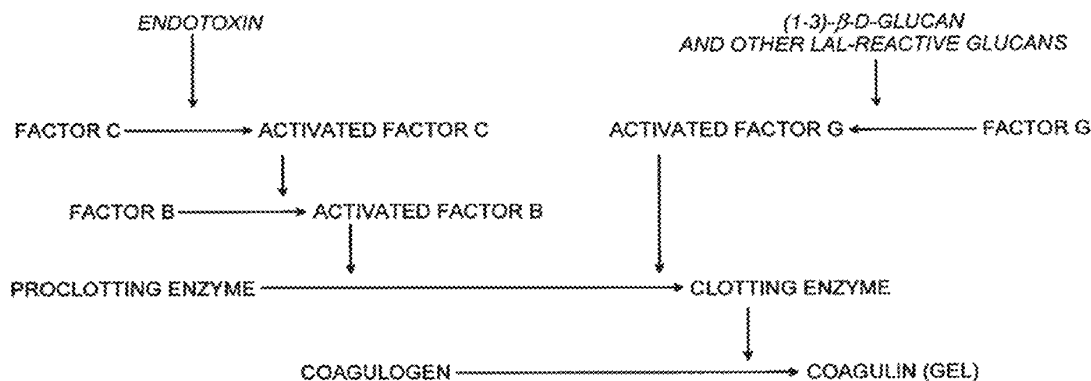




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(19) **United States**(12) **Patent Application Publication**
Wainwright et al.(10) **Pub. No.: US 2023/0258647 A1**(43) **Pub. Date: Aug. 17, 2023**(54) **HYBRID AMEBOCYTE LYSATE AND USES THEREOF**(71) Applicant: **Charles River Laboratories, Inc.**,
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(2013.01); **C12Y 304/21084** (2013.01); **C12Y**
304/21085 (2013.01); **C12Y 304/21086**
(2013.01)(57) **ABSTRACT**

The invention relates generally to hybrid amebocyte lysate compositions (including both native and recombinant components) and their use in detecting and/or quantifying endotoxin in a sample.

Specification includes a Sequence Listing.

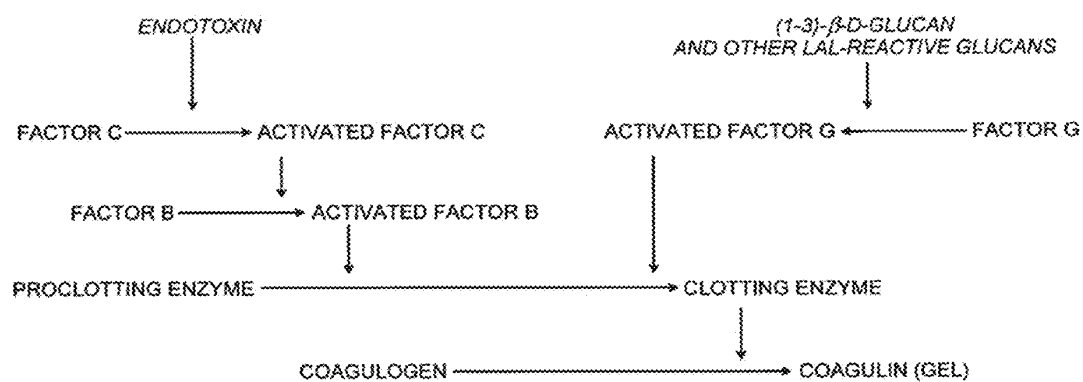


FIGURE 1

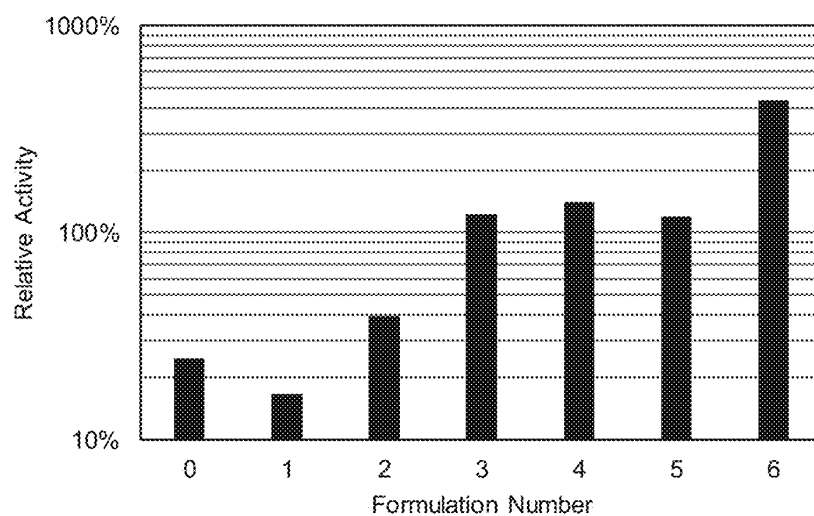


FIGURE 2

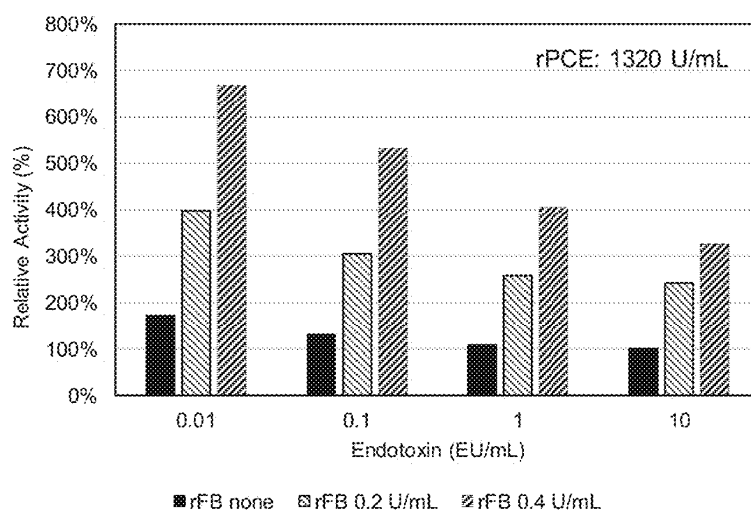


FIGURE 3

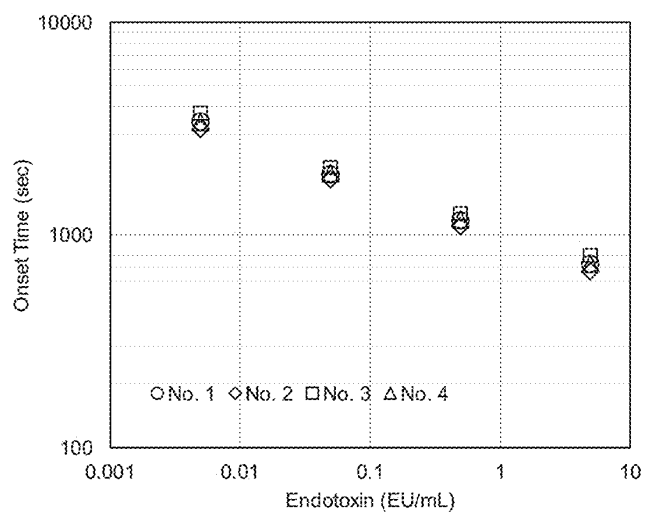


FIGURE 4

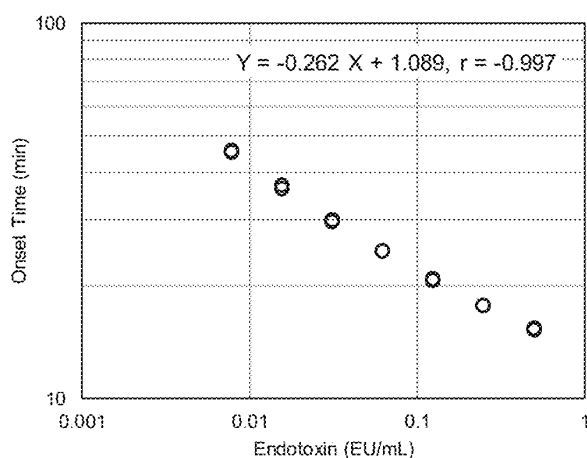


FIGURE 5A

Et _x (EU/mL)	Gel
0.0078	Negative
0.0078	Negative
0.0156	Positive
0.0156	Positive
0.03	Positive
0.03	Positive
0.06	Positive
0.06	Positive
0.125	Positive
0.125	Positive
0.25	Positive
0.25	Positive
0.5	Positive
0.5	Positive

FIGURE 5B

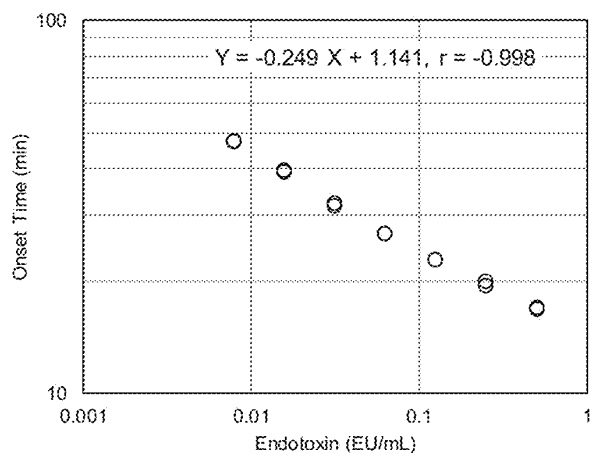


FIGURE 6A

Etx (EU/mL)	Gel
0.0078	Negative
0.0078	Negative
0.0156	Negative
0.0156	Negative
0.03	Negative
0.03	Negative
0.06	Negative
0.06	Negative
0.125	Negative
0.125	Negative
0.25	Negative
0.25	Negative
0.5	Negative
0.5	Negative

FIGURE 6B

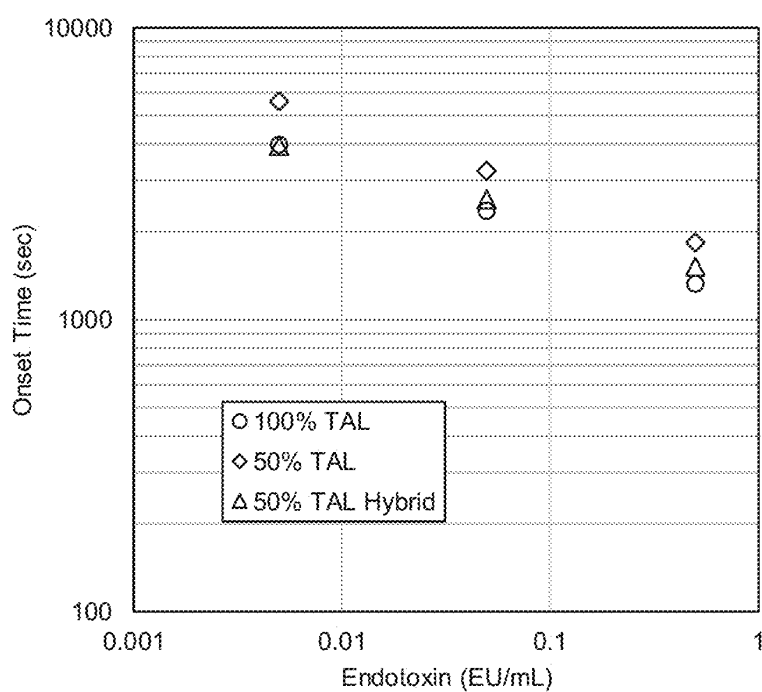


FIGURE 7

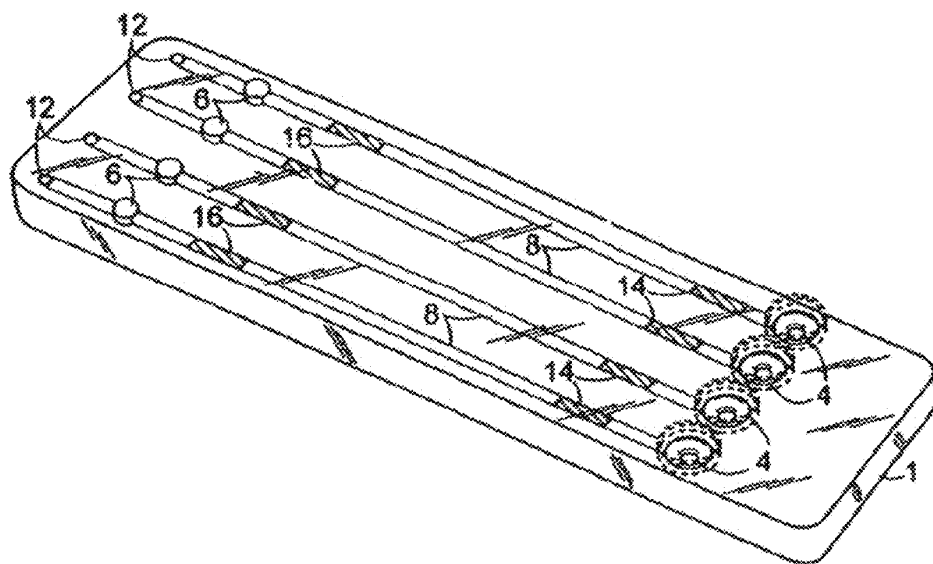


FIGURE 8A

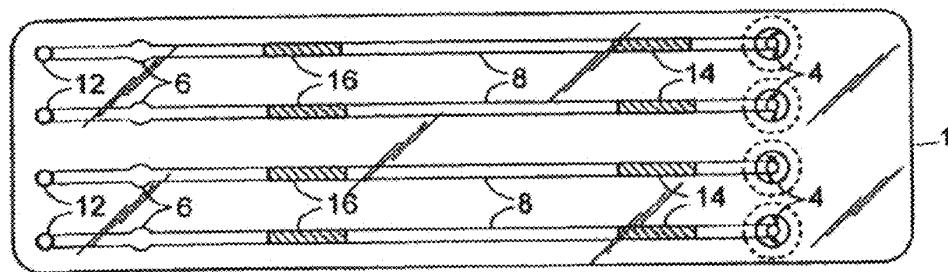


FIGURE 8B

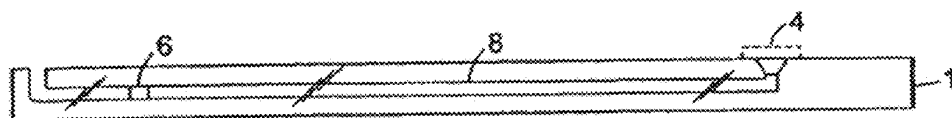


FIGURE 8C

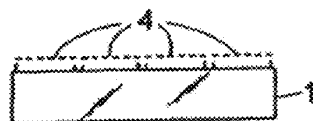


FIGURE 8D

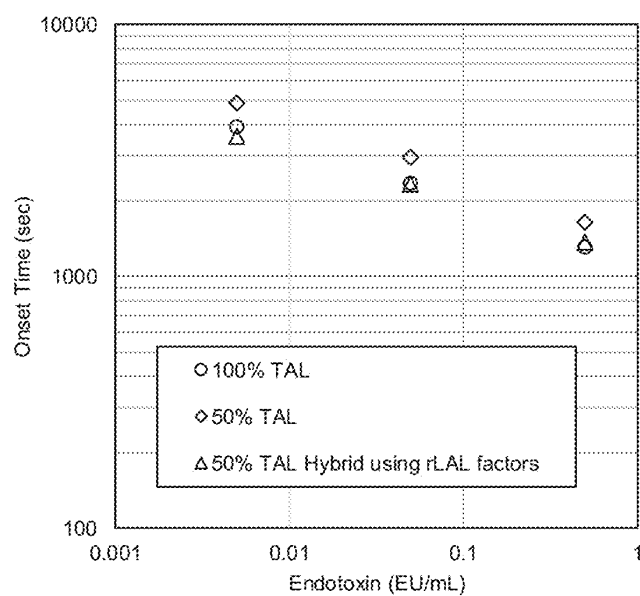


FIGURE 9

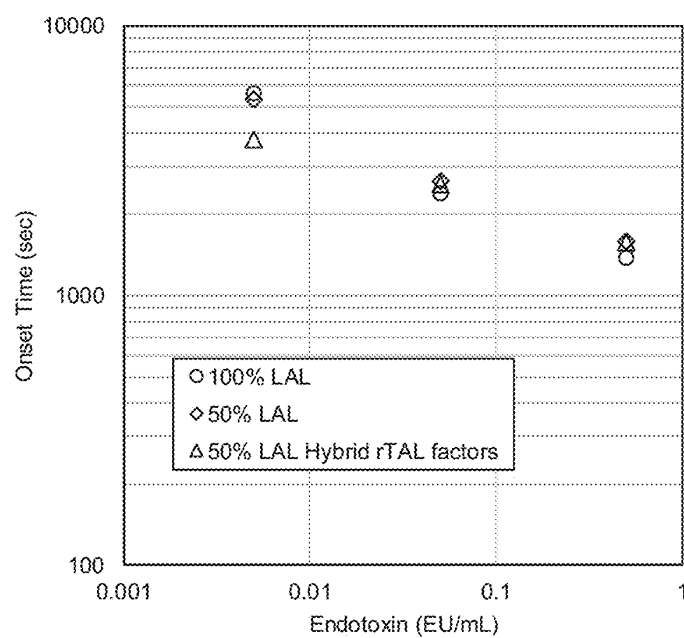


FIGURE 10

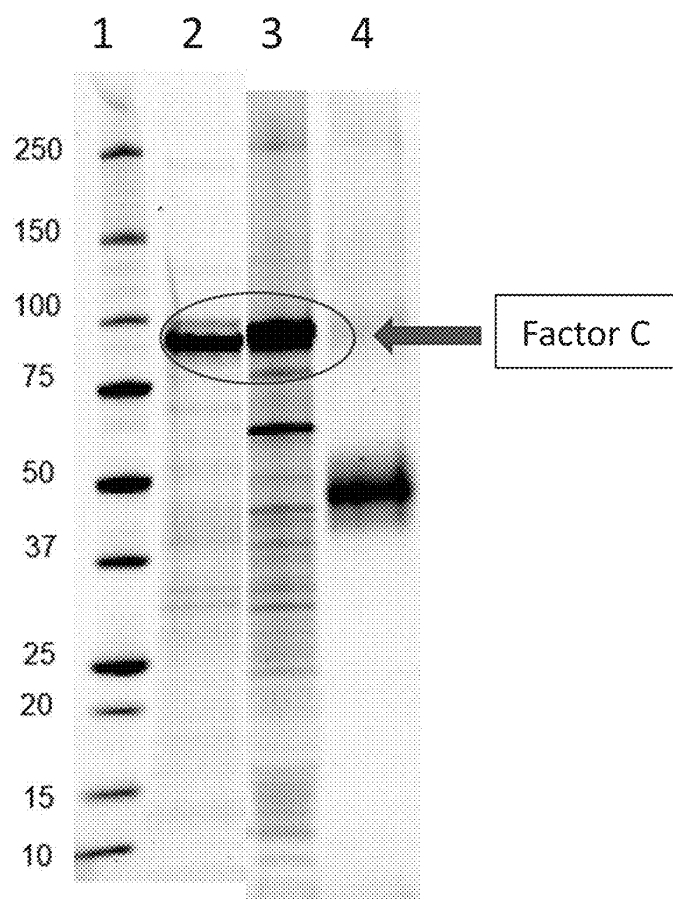


FIGURE 11

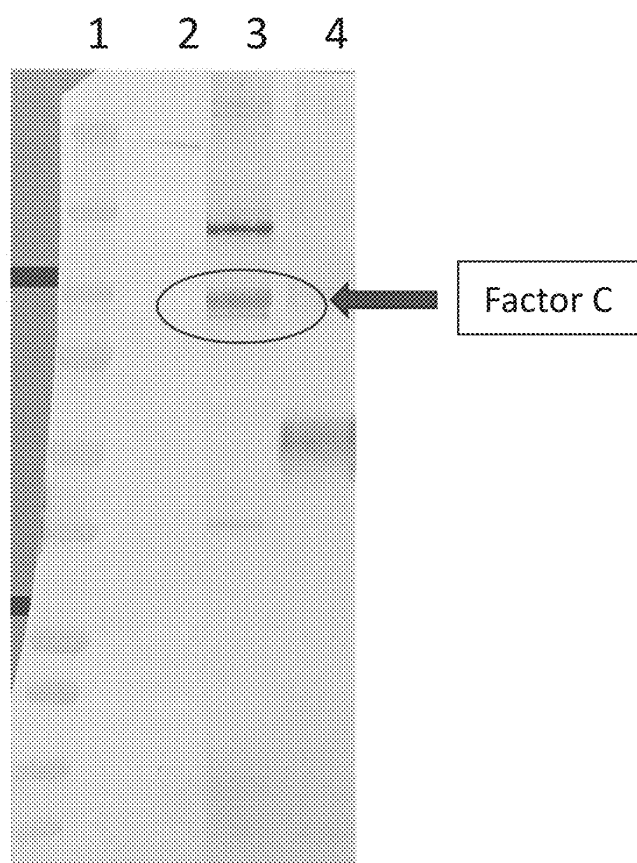


FIGURE 12

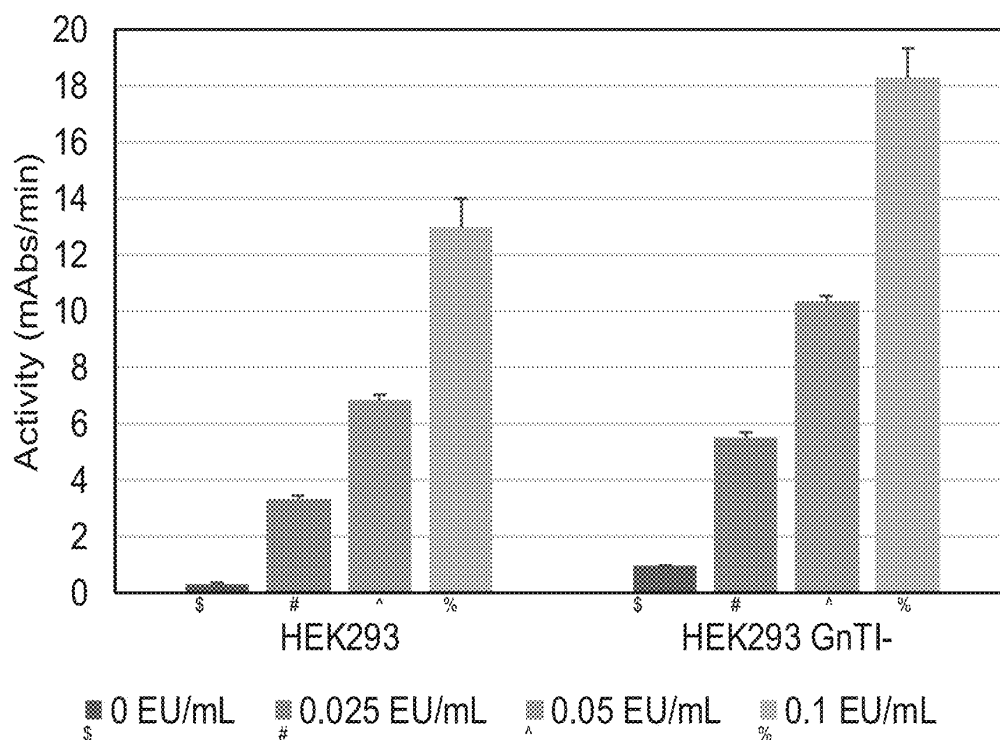


FIGURE 13

Host cells for Factor C	Endotoxin (EU/mL)	Absorbance change rate (av +/- SD, mAbs/min)
HEK293	0	0.32 +/- 0.02
	0.025	3.21 +/- 0.11
	0.05	6.77 +/- 0.13
	0.1	12.62 +/- 0.20
HEK293 GnTI-	0	0.97 +/- 0.02
	0.025	5.38 +/- 0.20
	0.05	10.43 +/- 0.15
	0.1	18.0 +/- 0.27

FIGURE 14

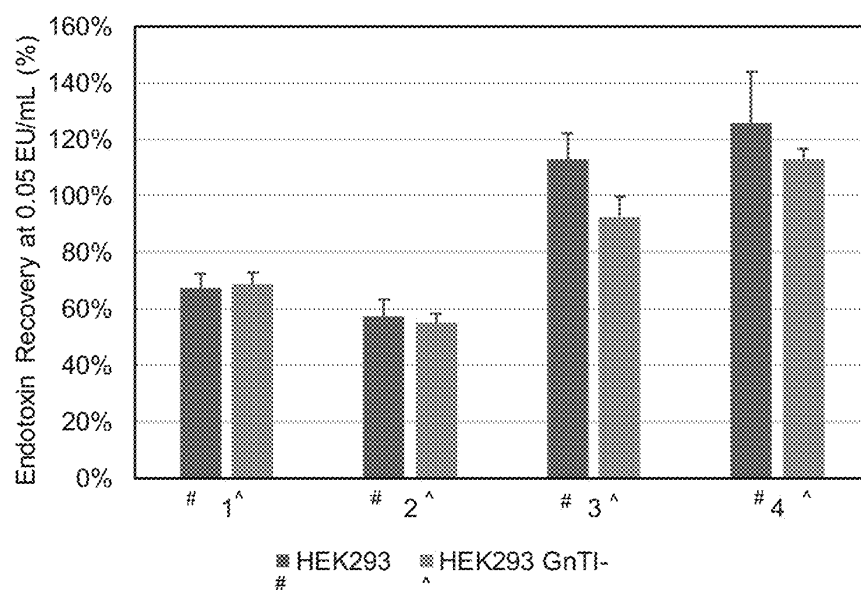


FIGURE 15

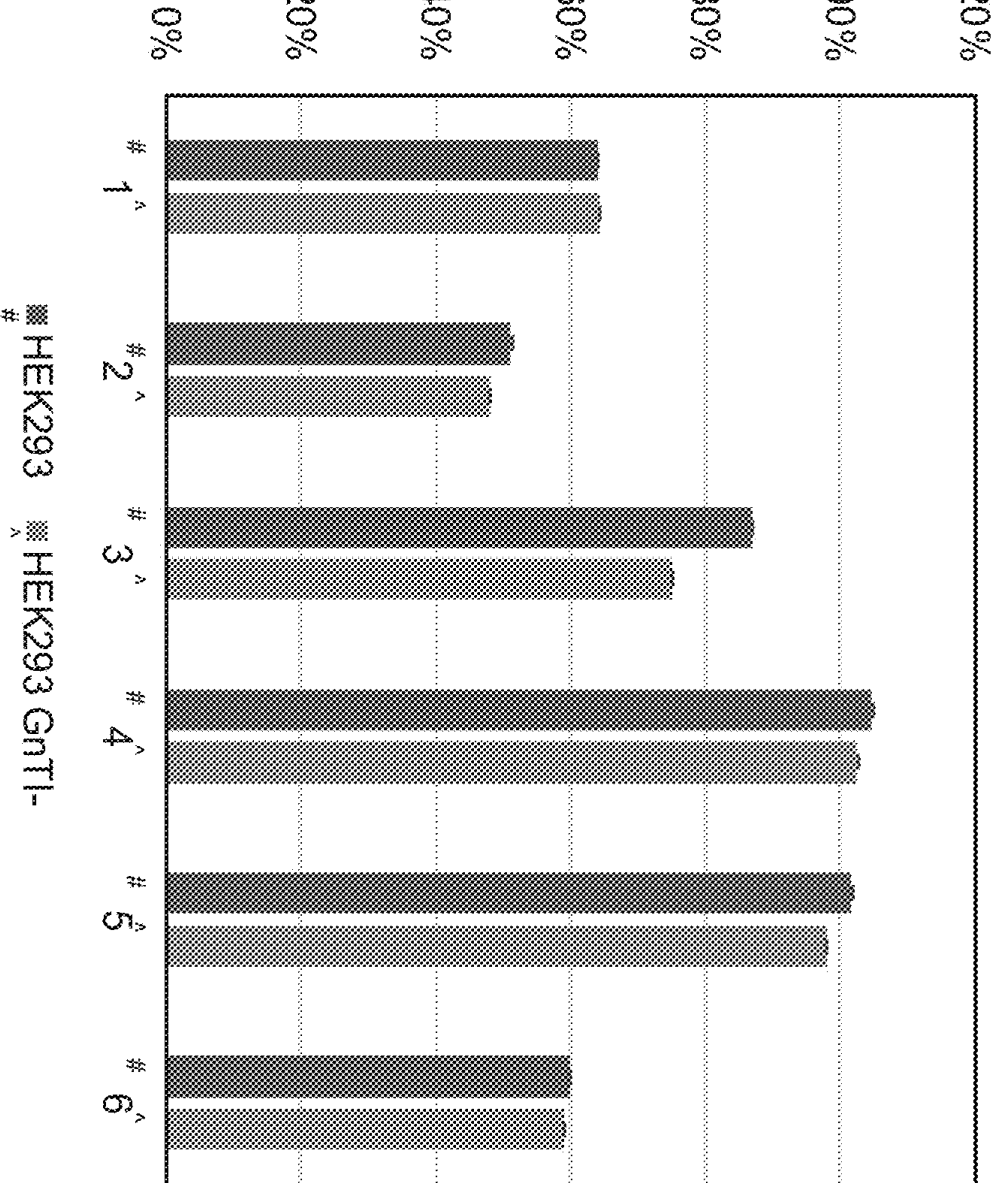


FIGURE 16

HYBRID AMEBOCYTE LYSATE AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates generally to methods and compositions for detecting and/or quantifying endotoxin in a sample. More particularly, the invention relates to hybrid amebocyte lysates (including native and recombinantly produced components) and their use for detecting and/or quantifying endotoxin in a sample.

BACKGROUND

[0002] Microbial contamination by, for example, Gram negative bacteria, may cause severe illness and, in some cases, even death in humans. Manufacturers in certain industries, for example, the pharmaceutical, medical device, and food industries, must meet exacting standards to verify that their products do not contain levels of microbial contaminants that would otherwise compromise the health of the recipient. These industries require frequent, accurate, and sensitive testing for the presence of such microbial contaminants to meet certain standards, for example, standards imposed by the United States Food and Drug Administration (USFDA) or Environmental Protection Agency. By way of example, the USFDA requires certain manufacturers of pharmaceuticals and invasive medical devices to establish that their products are free of detectable levels of Gram negative bacterial endotoxin.

[0003] Furthermore, when people become infected with Gram negative bacteria, the bacteria may produce and secrete fever-inducing bacterial endotoxins. Bacterial endotoxins can be dangerous and even deadly to humans. Symptoms of infection may range from fever, in mild cases, to death. In order to promptly initiate proper medical treatment, it usually is important to identify, as early as possible, the presence of an endotoxin and, if possible, the concentration of the endotoxin in the patient.

[0004] To date, a variety of assays have been developed to detect the presence and/or amount of endotoxin in a test sample. One family of assays use hemocyte lysates prepared from the hemolymph of crustaceans, for example, horseshoe crabs. These assays typically exploit, in one way or another, a clotting cascade that occurs when the hemocyte lysate is exposed to endotoxin. A currently preferred hemocyte lysate is amebocyte lysate (AL) produced from the hemolymph of a horseshoe crab, for example, *Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas*, and *Carcinoscorpius rotundicauda*.

[0005] These assays use blood that is harvested from horseshoe crabs, which has resulted in concerns over the ecological sustainability of this practice. However, to date, fully synthetic or recombinant amebocyte lysate reagents are not comparable in performance to native amebocyte lysate (Dubczak et al. (2021) EUR. J. PHRM. SCI. 159:105716). Accordingly, there exists a need for new reagents that reduce the burden on the horseshoe crab population while adequately detecting endotoxin at the same level of sensitivity and accuracy as naturally derived amebocyte lysates.

SUMMARY OF THE INVENTION

[0006] The invention is based, in part, upon the discovery that the addition of a recombinant factor B and/or a recombinant proclotting enzyme to a native amebocyte lysate, e.g.,

for example, a diluted native amebocyte lysate, to produce a hybrid amebocyte lysate can reduce the amount of the native amebocyte lysate required to detect endotoxin in a sample, while maintaining, or in certain instances even exceeding the sensitivity and/or activity of the native amebocyte lysate for endotoxin. Furthermore, it has been discovered that hybrid amebocyte lysates described herein are superior to fully recombinant reagents, and comparable or superior to native amebocyte lysates, at detecting naturally occurring endotoxins.

[0007] Accordingly, in one aspect, the invention provides a hybrid amebocyte lysate composition. The composition comprises a native horseshoe crab amebocyte lysate. The composition also comprises a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme.

[0008] In another aspect, the invention provides a hybrid amebocyte lysate composition. The composition comprises a native horseshoe crab factor C, a native horseshoe crab factor B, and a native horseshoe crab pro-clotting enzyme. The composition also comprises a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme.

[0009] In another aspect, the invention provides a hybrid amebocyte lysate composition. The composition comprises a horseshoe crab factor C, horseshoe crab factor B, and a horseshoe crab proclotting enzyme, wherein (a) the ratio of horseshoe crab factor B to horseshoe crab factor C is greater than the ratio of horseshoe crab factor B to horseshoe crab factor C in a native horseshoe crab amebocyte lysate, and/or (b) the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C is greater than the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C in a native horseshoe crab amebocyte lysate.

[0010] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the composition does not comprise a recombinant horseshoe crab factor C.

[0011] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the composition comprises from about 0.05 to about 1 U/mL, for example, from about 0.1 to about 0.5 U/mL, of recombinant horseshoe crab factor B.

[0012] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the composition comprises from 0.05 to about 2,000 U/mL, for example, from about 50 to about 200 U/mL, of recombinant proclotting enzyme.

[0013] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the composition has substantially the same or greater sensitivity in detecting endotoxin than native horseshoe crab amebocyte lysate. In certain embodiments, the composition retains substantially the same activity when stored at 4° C. for 3, 4, 5, 6, 7, or 8 hours.

[0014] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the horseshoe crab amebocyte lysate is a *Limulus polyphemus* amebocyte lysate. In certain embodiments, the horseshoe crab factor B is a *Limulus polyphemus* factor B. In certain embodiments, the horseshoe crab proclotting enzyme is a *Limulus polyphemus* proclotting enzyme. In certain embodiments, the horseshoe crab factor C is a *Limulus polyphemus* factor C.

[0015] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the horseshoe crab amebocyte lysate is a *Tachypleus* amebocyte lysate. In certain embodiments, the horseshoe crab factor B is a *Tachypleus* factor B. In certain embodiments, the horseshoe crab proclotting enzyme is a *Tachypleus* proclotting enzyme. In certain embodiments, the horseshoe crab factor C is a *Tachypleus* factor C.

[0016] In certain embodiments of any of the foregoing hybrid amebocyte lysate compositions, the recombinant horseshoe crab factor B and/or the recombinant horseshoe crab proclotting enzyme are expressed in a mammalian cell, for example, a Chinese hamster ovary (CHO) or human embryonic kidney (HEK) cell. In certain embodiments, the recombinant factor B and/or the recombinant proclotting enzyme has different glycosylation than the factor B and/or the proclotting enzyme present in the native horseshoe crab amebocyte lysate.

[0017] In another aspect, the invention provides a method for preparing an endotoxin detection reagent. The method comprises adding a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme to a native horseshoe crab amebocyte lysate.

[0018] In another aspect, the invention provides a method for increasing the endotoxin sensitivity of a native horseshoe crab amebocyte lysate. The method comprises adding to the native horseshoe crab amebocyte lysate a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme, thereby to increase the endotoxin sensitivity of the native horseshoe crab amebocyte lysate.

[0019] In another aspect, the invention provides a method for reducing the amount of a native horseshoe crab amebocyte lysate required to detect endotoxin. The method comprises: (i) diluting the native horseshoe crab amebocyte lysate; and (ii) adding to the diluted native horseshoe crab amebocyte lysate a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme to produce a hybrid amebocyte lysate.

[0020] In certain embodiments of any of the foregoing methods, the method does not comprise adding a recombinant horseshoe crab factor C to the native horseshoe crab amebocyte lysate or diluted native horseshoe crab amebocyte lysate.

[0021] In certain embodiments of any of the foregoing methods, the method comprises adding from about 0.05 to about 1 U/mL, e.g., from about 0.1 to about 0.5 U/mL, of recombinant horseshoe crab factor B to the native horseshoe crab amebocyte lysate or diluted native horseshoe crab amebocyte lysate.

[0022] In certain embodiments of any of the foregoing methods, the method comprises adding from 0.05 to about 2,000 U/mL, e.g., from about 50 to about 200 U/mL, of recombinant proclotting enzyme to the native horseshoe crab amebocyte lysate or diluted native horseshoe crab amebocyte lysate.

[0023] In certain embodiments of any of the foregoing methods, the method results in a composition that has substantially the same or greater sensitivity in detecting endotoxin than native horseshoe crab amebocyte lysate.

[0024] In certain embodiments of any of the foregoing methods, the horseshoe crab amebocyte lysate is a *Limulus polyphemus* amebocyte lysate. In certain embodiments, the horseshoe crab factor B is a *Limulus polyphemus* factor B. In certain embodiments, the horseshoe crab proclotting

enzyme is a *Limulus polyphemus* proclotting enzyme. In certain embodiments, the horseshoe crab factor C is a *Limulus polyphemus* factor C.

[0025] In certain embodiments of any of the foregoing methods, the recombinant horseshoe crab factor B and/or the recombinant horseshoe crab proclotting enzyme are expressed in a mammalian cell, for example, a Chinese hamster ovary (CHO) or human embryonic kidney (HEK) cell. In certain embodiments, the recombinant factor B and/or the recombinant proclotting enzyme has different glycosylation than the factor B and/or the proclotting enzyme present in the native horseshoe crab amebocyte lysate.

[0026] These and other aspects and features of the invention are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0027] The invention can be more completely understood with reference to the following drawings.

[0028] FIG. 1 is a schematic representation of the coagulation system present in amebocytes.

[0029] FIG. 2 is bar graph depicting relative activity of the indicated LAL reagents in a kinetic chromogenic assay (KCA).

[0030] FIG. 3 is bar graph depicting relative activity of the indicated LAL reagents in a kinetic chromogenic assay (KCA).

[0031] FIG. 4 is a line graph depicting onset time for the indicated LAL reagents as a function of endotoxin concentration in a kinetic chromogenic assay (KCA).

[0032] FIG. 5A is a line graph depicting onset time for a hybrid LAL reagent as a function of endotoxin concentration in a kinetic turbidometric assay (KTA). FIG. 5B is a chart depicting gel clot formation using a hybrid LAL reagent at the indicated endotoxin concentrations.

[0033] FIG. 6A is a line graph depicting onset time for a hybrid LAL reagent as a function of endotoxin concentration in a kinetic turbidometric assay (KTA). FIG. 6B is a chart depicting gel clot formation using a hybrid LAL reagent at the indicated endotoxin concentrations.

[0034] FIG. 7 is a line graph depicting onset time for the indicated TAL reagents as a function of endotoxin concentration in a kinetic chromogenic assay (KCA).

[0035] FIGS. 8A-8D are schematic illustrations in perspective view (FIG. 8A), top view (FIG. 8B), side view (FIG. 8C), and end view (FIG. 8D), of an exemplary cartridge useful in performing assays with the hybrid lysate described herein.

[0036] FIG. 9 is a line graph depicting onset time for the indicated reagents as a function of endotoxin concentration in a kinetic chromogenic assay (KCA).

[0037] FIG. 10 is a line graph depicting onset time for the indicated reagents as a function of endotoxin concentration in a kinetic chromogenic assay (KCA).

[0038] FIG. 11 is an image of a polyacrylamide electrophoresis gel where proteins are stained with SYPRO Orange. The bands of Factor C are circled. Lane 1 contains molecular weight markers. Lane 2 contains M21219-32 HEK293 GnTI- *Limulus* Factor C without sialic acid (2.7 µg loaded). Lane 3 contains M212199-28 HEK293 *Limulus* Factor C with sialic acid (3.0 µg loaded). Lane 4 contains Fetuin, a positive control sialic acid (10 µg loaded).

[0039] FIG. 12 is an image of a western blot stained with a sialic acid specific stain. Bands of Factor C are circled. Lane 1 contains molecular weight markers. Lane 2 contains M21219-32 HEK293 GnTI- *Limulus* Factor C without sialic acid (2.7 µg loaded). Lane 3 contains M21219-28 HEK293 *Limulus* Factor C with sialic acid (3.0 µg loaded). Lane 4 contains Fetuin, a positive control sialic acid (10 µg loaded).

[0040] FIG. 13 is a standard curve for recombinant LAL using US Reference Standard Endotoxin (RSE) at 0, 0.025, 0.05, and 0.1 EU/mL and recombinant Factor C (rFC) produced in both HEK293 and HEK2GnTI- cell lines (n=2).

[0041] FIG. 14 shows in Tabular format the absorbance change rates of RSE dilutions (n=3) of the standard curve provided in FIG. 13.

[0042] FIG. 15 is a bar graph showing residual activity (endotoxin recovery) of endotoxin in salt solutions (n=4) when spiked with endotoxin at a concentration of 0.05 EU/mL. rLALs containing rFCs expressed in HEK293 and GnTI- cells were used. Sample 1 contained 1.25% (214 mM) NaCl. Sample 2 contained 2.5% (428 mM) NaCl. Sample 3 contained 16 mM MgSO₄. Sample 4 contained 2.5 mM CaCl₂.

[0043] FIG. 16 is a bar graph showing the residual activity (endotoxin recovery) of endotoxin in salt solutions (n=2) when spiked with endotoxin at a concentration of 0.05 EU/mL. rLALs containing rFCs expressed in HEK293 and GnTI- cells were used. Sample 1 contained 1.25% (214 mM) NaCl; sample 2 contained 2.5% (428 mM) NaCl; sample 3 contained 16 mM MgSO₄; sample 4 contained 2.5 mM CaCl₂; sample 5 contained 21 mM sodium citrate; and sample 6 contained 52 mM sodium hydrogen carbonate.

DETAILED DESCRIPTION

[0044] The invention is based, in part, upon the discovery that addition of a recombinant factor B and/or a recombinant proclotting enzyme to a native amebocyte lysate to produce a hybrid amebocyte lysate can reduce the amount of the native amebocyte lysate required to detect endotoxin in a sample, while maintaining, or in certain instances even increasing the sensitivity and/or activity of the amebocyte lysate. Furthermore, it has been discovered that hybrid amebocyte lysates described herein are superior to fully recombinant reagents, and comparable or superior to native amebocyte lysates, at detecting naturally occurring endotoxins.

[0045] Various features and aspects of the invention are discussed in more detail below.

I. Hybrid Amebocyte Lysate

[0046] The invention relates, in part, to hybrid amebocyte lysate compositions including one or more native components (e.g., a native horseshoe crab amebocyte lysate, a native horseshoe crab factor C, a native horseshoe crab factor B, and/or a native horseshoe crab pro-clotting enzyme) and one or more recombinant components (e.g., a recombinant horseshoe crab factor B, a recombinant horseshoe crab proclotting enzyme, and/or a recombinant horseshoe crab factor C).

[0047] In one aspect, the invention provides a hybrid amebocyte lysate composition comprising: (i) a native horseshoe crab amebocyte lysate, and (ii) a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme.

[0048] In one aspect, the invention provides a hybrid amebocyte lysate composition comprising: (i) a native horseshoe crab factor C, (ii) a native horseshoe crab factor B, (iii) a native horseshoe crab pro-clotting enzyme, and (iv) a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme.

[0049] In one aspect, the invention provides an amebocyte lysate composition comprising: (i) a horseshoe crab factor C, (ii) horseshoe crab factor B, and (iii) a horseshoe crab proclotting enzyme, wherein (a) the ratio of horseshoe crab factor B to horseshoe crab factor C is greater than the ratio of horseshoe crab factor B to horseshoe crab factor C in a native horseshoe crab amebocyte lysate, and/or (b) the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C is greater than the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C in a native horseshoe crab amebocyte lysate. It is understood that the ratio of horseshoe crab factor B to horseshoe crab factor C or the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C may refer to a ratio of enzymatic activity. Enzymatic activity may be measured by a method known in the art (e.g., as described in Example 1 hereinbelow). Alternatively, the ratio of horseshoe crab factor B to horseshoe crab factor C or the ratio of horseshoe crab proclotting enzyme to horseshoe crab factor C may refer to a ratio of protein quantity. Protein quantity may be measured by any method known in the art, including for example, Western blot, ELISA, or ultraviolet (UV) absorption at 280 nm.

[0050] In certain embodiments, a hybrid amebocyte lysate composition does not contain recombinant horseshoe crab factor C.

[0051] In certain embodiments, a hybrid amebocyte lysate composition has substantially the same or greater sensitivity for a microbial contaminant (e.g., endotoxin) as a corresponding native horseshoe crab amebocyte lysate. For example, the hybrid amebocyte lysate composition may have at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 175%, at least 200%, at least 225%, at least 250%, at least 300%, at least 350%, or at least 400% of the sensitivity of the corresponding native horseshoe crab amebocyte lysate. Amebocyte lysate sensitivity may be assayed by any method known in the art, including, for example, a kinetic chromogenic assay method (KCA), as described in Example 2 hereinbelow.

[0052] In certain embodiments, a hybrid amebocyte lysate composition containing less crude lysate than a reference native amebocyte lysate (e.g., containing 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the crude lysate of a reference native amebocyte lysate) has the substantially the same or greater sensitivity for a microbial contaminant (e.g., endotoxin) as the reference native horseshoe crab amebocyte lysate. For example, the hybrid amebocyte lysate composition may have at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 175%, at least 200%, at least 225%, at least 250%, at least 300%, at least 350%, or at least 400% of the sensitivity of the reference native horseshoe crab amebocyte lysate. In certain embodiments, the hybrid amebocyte lysate composition is substantially the same as the

reference native amebocyte lysate but for (i) the amount of crude lysate present, and (ii) the presence or absence of any recombinant proteins. Amebocyte lysate sensitivity may be assayed by any method known in the art, including, for example, a kinetic chromogenic assay method (KCA), as described in Example 2 hereinbelow.

[0053] In certain embodiments, a hybrid amebocyte lysate composition retains substantially the same activity when stored at 4° C. for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 hours, or 3, 4, 5, 6, 7, or 8 hours.

A. Native Horseshoe Crab Amebocyte Lysate

[0054] As shown in FIG. 1, the coagulation system of hemolymph, like the mammalian blood coagulation system, comprises at least two coagulation cascades that include an endotoxin-mediated pathway (the Factor C pathway) and a (1→3)-B-D glucan-mediated pathway (the Factor G pathway).

[0055] When bacterial endotoxin is contacted with LAL, the endotoxin initiates a series of enzymatic reactions, referred to in the art as the Factor C pathway, that can involve three serine protease zymogens called Factor C, Factor B, and pro-clotting enzyme (see, FIG. 1). Briefly, upon exposure to endotoxin, the endotoxin-sensitive factor, Factor C, is activated. Activated Factor C thereafter hydrolyzes and activates Factor B, whereupon activated Factor B activates proclotting enzyme to produce clotting enzyme. The clotting enzyme thereafter hydrolyzes specific sites, for example, Arg¹⁸-Thr¹⁹ and Arg⁴⁶-Gly⁴⁷ of coagulogen, an invertebrate, fibrinogen-like clottable protein, to produce a coagulin gel. See, for example, U.S. Pat. No. 5,605,806.

[0056] (1→3)-B-D glucans and other amebocyte lysate reactive glucans, produced by microorganisms such as yeasts and molds, can also activate the clotting cascade of amebocyte lysates, through a different enzymatic pathway, referred to in the art as the Factor G pathway (see, FIG. 1). Factor G is a serine protease zymogen that becomes activated by (1→3)-β-D glucan or other LAL reactive glucans. Upon exposure to (1→3)-β-D glucan, for example, Factor G is activated to produce activated Factor G. Activated Factor G thereafter converts the proclotting enzyme into clotting enzyme, whereupon the clotting enzyme converts coagulogen into coagulin.

[0057] As used herein, the term “native horseshoe crab amebocyte lysate” is understood to mean any lysate or fraction thereof (e.g., the components of a factor C mediated cascade) produced by the lysis, extrusion, or extraction of the cellular contents from amebocytes extracted from a horseshoe crab. Under certain circumstances, the native amebocyte lysate comprises the naturally occurring components of an enzymatic cascade (e.g., as shown in FIG. 1) produced, for example, by the lysis, extrusion, or extraction of the cellular contents from amebocytes extracted from a horseshoe crab. A native horse shoe crab amebocyte lysate, which does not contain a recombinant zymogen or functional fragment thereof (e.g., recombinant factor B, recombinant factor C or recombinant proclotting enzyme) can be used to produce a hybrid lysate described herein, by supplementation of the native amebocyte lysate with, for example, a recombinant zymogen or functional fragment thereof (e.g., recombinant factor B, recombinant factor C or recombinant proclotting enzyme). Depending upon the components present in, or admixed with, the lysate it may produce a clot in the presence of an endotoxin, for example, a Gram negative

bacterial endotoxin and/or a glucan, for example, a (1→3)-β-D glucan, produced by a yeast or a mold. Preferred amebocyte lysates can be derived from horseshoe crabs belonging to the *Limulus* genus, for example, *Limulus polyphemus*, the *Tachypleus* genus, for example, *Tachypleus tridentatus* and *Tachypleus gigas*, and the *Carcinoscorpius* genus, for example, *Carcinoscorpius rotundicauda*.

[0058] In certain embodiments, a native horseshoe crab amebocyte lysate includes each of a native factor C, a native factor B, and a native proclotting enzyme.

B. Methods of Making Native Horseshoe Crab Amebocyte Lysate

[0059] Crude lysates may be produced using the procedure described in Levin et al. (1968) THROMB. DIATH. HAEMORRH. 19: 186, with modification, or in Prior 1990 “Clinical Applications of the *Limulus* Amebocyte Lysate Test” CRC PRESS 28-36 and 159-166, and in U.S. Pat. No. 4,322,217. Other lysates may include those, for example, described in U.S. Pat. Nos. 6,270,982 and 6,391,570. In certain embodiments, a crude lysate is produced as described in Example 1 hereinbelow.

[0060] It is possible to produce an endotoxin-specific lysate by removing Factor G activity from the lysate, such as the Factor G depleted lysates produced by the methods described in U.S. Pat. Nos. 6,391,570 and 6,270,982. Also, it is contemplated that lysates may be depleted of Factor G activity by the addition of certain inhibitors or modulators of Factor G activity, for example, certain detergents, saccharides, polysaccharides, and other reagents described in U.S. Pat. Nos. 5,155,032; 5,179,006; 5,318,893; 5,474,984; 5,641,643; and 6,270,982. An endotoxin-specific lysate is a lysate capable of reacting with a bacterial endotoxin but in which the reactivity to (1→3)-B-D glucan has been depleted by at least 80%, more preferably by at least 90%, and more preferably by at least 95% relative to the crude lysate from which the endotoxin-specific lysate was prepared.

[0061] Methods for enhancing the sensitivity of hemocyte lysate for endotoxin, for example, may include, without limitation, aging the crude hemocyte lysate, adjusting pH, adjusting the concentration of divalent cations, adjusting the concentration of coagulogen, chloroform extraction, and the addition of serum albumin, biocompatible buffers and/or biological detergents.

[0062] As will be apparent to one of ordinary skill, divalent metal salts, which are known to promote activation of the pro-clotting enzyme of hemocyte lysate, as well as buffers to avoid extremes of pH that could inactivate the clotting enzyme preferably are included in the lysate. Any of the buffers and salts that are understood in the art to be compatible with the amebocyte lysate system may be used. Typical formulation additives may include, without limitation, about 100-300 mM NaCl, about 10-100 mM divalent cations (e.g., Mg²⁺), biocompatible buffers, e.g., Tris (tris (hydroxy)aminomethane), to give a final pH of about 6.0 to about 8.0, and, if the lysate is to be freeze dried, then sugars, e.g., mannitol or dextran. It is contemplated that the choice of appropriate formulation additives may also be determined by routine experimentation.

C. Factor B

[0063] As used herein, the term “factor B” refers to a zymogen, or a functional fragment thereof, that is capable of

being activated upon cleavage by a factor C, and is capable of cleaving (e.g., enzymatically cleaving) a proclotting enzyme to form an active clotting enzyme. The term factor B includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type factor B sequence, and/or fusion proteins or conjugates including a factor B. As used herein, the term “functional fragment” of a factor B refers to fragment of a full-length factor B that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring factor B. Factor B enzymatic activity may be assayed by any method known in the art, including, for example, by measuring cleavage of the chromogenic substrate H-D-Leu-Thr-Arg-pNA, for example, as described in Example 1 hereinbelow. In certain embodiments, the functional fragment comprises at least 100, 150, 200, 250, 300, 350, 360, 370, 380, or 390 consecutive amino acids present in a full-length, naturally occurring factor B.

[0064] It is contemplated that any horseshoe crab factor B, for example, a *Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas*, or *Carcinoscorpius rotundicauda* factor B, may be used in the practice of the invention.

[0065] A DNA sequence encoding an exemplary *Limulus polyphemus* factor B is depicted in SEQ ID NO: 4. Exemplary *Limulus polyphemus* factor B amino acid sequences are depicted in SEQ ID NOs: 5 and 6. SEQ ID NO: 5 is the mature form whereas SEQ ID NO: 6 includes the signal sequence as residues 1-25. A DNA sequence encoding an exemplary *Tachypleus tridentatus* factor B is depicted in SEQ ID NO: 13. Exemplary *Tachypleus tridentatus* factor B amino acid sequences are depicted in SEQ ID NOs: 14 and 15. SEQ ID NO: 14 is the mature form whereas SEQ ID NO: 15 includes the signal sequence as residues 1-22. In certain embodiments, a factor B comprises the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 15 or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identity to the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 15.

[0066] Sequence identity may be determined in various ways that are within the skill in the art, e.g., using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin et al. (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul (1993) J. MOL. EVOL. 36, 290-300; Altschul et al. (1997) NUCLEIC ACIDS RES. 25:3389-3402, incorporated by reference) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases, see Altschul et al. (1994) NATURE GENETICS 6:119-129, which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp,

blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff et al. (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (e.g.: -G, Cost to open gap [Integer]: default=5 for nucleotides/11 for proteins; -E, Cost to extend gap [Integer]: default=2 for nucleotides/1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default=-3; -r, reward for nucleotide match [Integer]: default=1; -e, expect value [Real]: default=10; -W, wordsize [Integer]: default=11 for nucleotides/28 for megablast/3 for proteins; -y, Dropoff (X) for blast extensions in bits: default=20 for blastn/7 for others; -X, X dropoff value for gapped alignment (in bits): default=15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, e.g., Blosum62 matrix and Gap Opening Penalty=10 and Gap Extension Penalty=0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0067] In certain embodiments, the factor B comprises a conservative substitution relative to a wild-type factor B sequence or a factor B sequence disclosed herein. As used herein, the term “conservative substitution” refers to a substitution with a structurally similar amino acid. For example, conservative substitutions may include those within the following groups: Ser and Cys; Leu, Ile, and Val; Glu and Asp; Gln and Asn; Lys, Arg and His; Phe, Tyr, and Trp. Conservative substitutions may also be defined by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (e.g., BLOSUM 62 matrix), or the PAM substitution matrix (e.g., the PAM 250 matrix).

[0068] In certain embodiments, the factor B is a recombinant factor B. As used herein, when referring to a protein or polypeptide, the term “recombinant” refers to a protein or polypeptide which is produced by recombinant nucleic acid, e.g., recombinant DNA, techniques, wherein generally, DNA or other nucleic acid encoding the protein or polypeptide is inserted into a suitable expression vector which is in turn introduced in to a host cell to produce the heterologous protein within the host cell.

[0069] In certain embodiments, the factor B is a native factor B. As used herein, when referring to a protein or polypeptide (e.g., native factor B), the term “native” refers to a protein or polypeptide derived from a natural source as opposed to a protein or polypeptide produced by using recombinant means, e.g., recombinant DNA technologies. A native protein or polypeptide (e.g., factor B) may be isolated. As used herein, term “isolated” refers to molecules or biological or cellular materials that are in an environment that is different from their naturally occurring environment. The term “isolated” encompasses a nucleic acid, such as DNA or RNA, or protein or polypeptide, or cell or cellular organelle, or tissue or organ, separated from other DNAs

RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, that are present in a natural source.

[0070] A contemplated composition may comprise, for example, from about 0.01 to about 5 U/mL, from about 0.01 to about 3 U/mL, from about 0.01 to about 1 U/mL, from about 0.01 to about 0.9 U/mL, from about 0.01 to about 0.8 U/mL, from about 0.01 to about 0.7 U/mL, from about 0.01 to about 0.6 U/mL, from about 0.01 to about 0.5 U/mL, from about 0.01 to about 0.4 U/mL, from about 0.01 to about 0.3 U/mL, from about 0.01 to about 0.2 U/mL, from about 0.01 to about 0.1 U/mL, from about 0.01 to about 0.075 U/mL, from about 0.01 to about 0.05 U/mL, from about 0.05 to about 5 U/mL, from about 0.05 to about 3 U/mL, from about 0.05 to about 1 U/mL, from about 0.05 to about 0.9 U/mL, from about 0.05 to about 0.8 U/mL, from about 0.05 to about 0.7 U/mL, from about 0.05 to about 0.6 U/mL, from about 0.05 to about 0.5 U/mL, from about 0.05 to about 0.4 U/mL, from about 0.05 to about 0.3 U/mL, from about 0.05 to about 0.2 U/mL, from about 0.05 to about 0.1 U/mL, from about 0.05 to about 0.075 U/mL, from about 0.075 to about 5 U/mL, from about 0.075 to about 3 U/mL, from about 0.075 to about 1 U/mL, from about 0.075 to about 0.9 U/mL, from about 0.075 to about 0.8 U/mL, from about 0.075 to about 0.7 U/mL, from about 0.075 to about 0.6 U/mL, from about 0.075 to about 0.5 U/mL, from about 0.075 to about 0.4 U/mL, from about 0.075 to about 0.3 U/mL, from about 0.075 to about 0.2 U/mL, from about 0.075 to about 0.1 U/mL, from about 0.1 to about 5 U/mL, from about 0.1 to about 3 U/mL, from about 0.1 to about 1 U/mL, from about 0.1 to about 0.9 U/mL, from about 0.1 to about 0.8 U/mL, from about 0.1 to about 0.7 U/mL, from about 0.1 to about 0.6 U/mL, from about 0.1 to about 0.5 U/mL, from about 0.1 to about 0.4 U/mL, from about 0.1 to about 0.3 U/mL, from about 0.1 to about 0.2 U/mL, from about 0.2 to about 5 U/mL, from about 0.2 to about 3 U/mL, from about 0.2 to about 1 U/mL, from about 0.2 to about 0.9 U/mL, from about 0.2 to about 0.8 U/mL, from about 0.2 to about 0.7 U/mL, from about 0.2 to about 0.6 U/mL, from about 0.2 to about 0.5 U/mL, from about 0.2 to about 0.4 U/mL, from about 0.2 to about 0.3 U/mL, from about 0.3 to about 5 U/mL, from about 0.3 to about 3 U/mL, from about 0.3 to about 1 U/mL, from about 0.3 to about 0.9 U/mL, from about 0.3 to about 0.8 U/mL, from about 0.3 to about 0.7 U/mL, from about 0.3 to about 0.6 U/mL, from about 0.3 to about 0.5 U/mL, from about 0.3 to about 0.4 U/mL, from about 0.4 to about 5 U/mL, from about 0.4 to about 3 U/mL, from about 0.4 to about 1 U/mL, from about 0.4 to about 0.9 U/mL, from about 0.4 to about 0.8 U/mL, from about 0.4 to about 0.7 U/mL, from about 0.4 to about 0.6 U/mL, from about 0.4 to about 0.5 U/mL, from about 0.5 to about 5 U/mL, from about 0.5 to about 3 U/mL, from about 0.5 to about 1 U/mL, from about 0.5 to about 0.9 U/mL, from about 0.5 to about 0.8 U/mL, from about 0.5 to about 0.7 U/mL, from about 0.5 to about 0.6 U/mL, from about 0.6 to about 5 U/mL, from about 0.6 to about 3 U/mL, from about 0.6 to about 1 U/mL, from about 0.6 to about 0.9 U/mL, from about 0.6 to about 0.8 U/mL, from about 0.6 to about 0.7 U/mL, from about 0.7 to about 5 U/mL, from about 0.7 to about 3 U/mL, from about 0.7 to about 1 U/mL, from about 0.7 to about 0.9 U/mL, from about 0.7 to about 0.8 U/mL, from about 0.8 to about 5 U/mL, from about 0.8 to about 3 U/mL, from about 0.8 to about 1 U/mL, from about 0.8 to about 0.9 U/mL, from about 0.9 to about 5 U/mL,

from about 0.9 to about 3 U/mL, from about 0.9 to about 1 U/mL, from about 1 to about 5 U/mL, from about 1 to about 3 U/mL, or from about 3 to about 5 U/mL of factor B (e.g., recombinant factor B). Factor B enzymatic activity may be assayed by any method known in the art, including, for example, by measuring cleavage of the chromogenic substrate H-D-Leu-Thr-Arg-pNA, for example, as described in Example 1 herein. As used herein one unit (U) of factor B activity is defined as the amount of enzyme that catalyzes the conversion of 1 micromole of a substrate (e.g., H-D-Leu-Thr-Arg-pNA) per minute.

D. Proclotting Enzyme

[0071] As used herein, the term “proclotting enzyme” refers to a zymogen, or a functional fragment thereof, that is capable of being activated upon cleavage by an activated factor B, and is capable of cleaving (e.g., enzymatically cleaving) coagulogen to form coagulin. The term proclotting enzyme includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type proclotting enzyme sequence, and/or fusion proteins or conjugates including a proclotting enzyme. As used herein, the term “functional fragment” of a proclotting enzyme refers to fragment of a full-length proclotting enzyme that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring proclotting enzyme. Proclotting enzyme enzymatic activity may be assayed by any method known in the art, including, for example, by measuring cleavage of the chromogenic substrate Ac-Ilu-Glu-Gly-Arg-pNA, for example, as described in Example 1 herein. In certain embodiments, the functional fragment comprises at least 100, 150, 200, 250, 300, 320, 330, 340, 350, 360, or 370 consecutive amino acids present in a full-length, naturally occurring proclotting enzyme.

[0072] It is contemplated that any horseshoe crab factor proclotting enzyme, for example, a *Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas*, or *Carcinoscorpius rotundicauda* proclotting enzyme, may be used in the practice of the invention.

[0073] A DNA sequence encoding an exemplary *Limulus polyphemus* proclotting enzyme is depicted in SEQ ID NO: 7. Exemplary *Limulus polyphemus* proclotting enzyme amino acid sequences are depicted in SEQ ID NOs: 8 and 9. SEQ ID NO: 8 is the mature form whereas SEQ ID NO: 9 includes the signal sequence as residues 1-28. A DNA sequence encoding an exemplary *Tachypleus tridentatus* proclotting enzyme is depicted in SEQ ID NO: 16. Exemplary *Tachypleus tridentatus* proclotting enzyme amino acid sequences are depicted in SEQ ID NOs: 17 and 18. SEQ ID NO: 17 is the mature form whereas SEQ ID NO: 18 includes the signal sequence as residues 1-21. In certain embodiments, a proclotting enzyme comprises the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 17, or SEQ ID NO: 18, or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identity to the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 17, or SEQ ID NO: 18. In certain embodiments, the proclotting enzyme com-

prises a conservative substitution relative to a wild-type proclotting enzyme sequence or a proclotting enzyme sequence disclosed herein.

[0074] In certain embodiments, the proclotting enzyme is a recombinant proclotting enzyme. In certain embodiments, the proclotting enzyme is a native proclotting enzyme, e.g., an isolated native proclotting enzyme.

[0075] A contemplated composition may comprise, for example, from about 0.01 to about 3,000 U/mL, from about 0.01 to about 2,500 U/mL, from about 0.01 to about 2,000 U/mL, from about 0.01 to about 1,500 U/mL, from about 0.01 to about 1,000 U/mL, from about 0.01 to about 500 U/mL, from about 0.01 to about 400 U/mL, from about 0.01 to about 300 U/mL, from about 0.01 to about 200 U/mL, from about 0.01 to about 100 U/mL, from about 0.01 to about 50 U/mL, from about 0.01 to about 20 U/mL, from about 0.01 to about 10 U/mL, from about 0.01 to about 5 U/mL, from about 0.01 to about 2 U/mL, from about 0.01 to about 1 U/mL, from about 0.01 to about 0.5 U/mL, from about 0.01 to about 0.1 U/mL, from about 0.1 to about 3,000 U/mL, from about 0.1 to about 2,500 U/mL, from about 0.1 to about 2,000 U/mL, from about 0.1 to about 1,500 U/mL, from about 0.1 to about 1,000 U/mL, from about 0.1 to about 500 U/mL, from about 0.1 to about 400 U/mL, from about 0.1 to about 300 U/mL, from about 0.1 to about 200 U/mL, from about 0.1 to about 100 U/mL, from about 0.1 to about 50 U/mL, from about 0.1 to about 20 U/mL, from about 0.1 to about 10 U/mL, from about 0.1 to about 5 U/mL, from about 0.1 to about 2 U/mL, from about 0.1 to about 1 U/mL, from about 0.1 to about 0.5 U/mL, from about 0.5 to about 3,000 U/mL, from about 0.5 to about 2,500 U/mL, from about 0.5 to about 2,000 U/mL, from about 0.5 to about 1,500 U/mL, from about 0.5 to about 1,000 U/mL, from about 0.5 to about 500 U/mL, from about 0.5 to about 400 U/mL, from about 0.5 to about 300 U/mL, from about 0.5 to about 200 U/mL, from about 0.5 to about 100 U/mL, from about 0.5 to about 50 U/mL, from about 0.5 to about 20 U/mL, from about 0.5 to about 10 U/mL, from about 0.5 to about 5 U/mL, from about 0.5 to about 2 U/mL, from about 0.5 to about 1 U/mL, from about 1 to about 3,000 U/mL, from about 1 to about 2,500 U/mL, from about 1 to about 2,000 U/mL, from about 1 to about 1,500 U/mL, from about 1 to about 1,000 U/mL, from about 1 to about 500 U/mL, from about 1 to about 400 U/mL, from about 1 to about 300 U/mL, from about 1 to about 200 U/mL, from about 1 to about 100 U/mL, from about 1 to about 50 U/mL, from about 1 to about 20 U/mL, from about 1 to about 10 U/mL, from about 1 to about 5 U/mL, from about 1 to about 2 U/mL, from about 2 to about 3,000 U/mL, from about 2 to about 2,500 U/mL, from about 2 to about 2,000 U/mL, from about 2 to about 1,500 U/mL, from about 2 to about 1,000 U/mL, from about 2 to about 500 U/mL, from about 2 to about 400 U/mL, from about 2 to about 300 U/mL, from about 2 to about 200 U/mL, from about 2 to about 100 U/mL, from about 2 to about 50 U/mL, from about 2 to about 20 U/mL, from about 2 to about 10 U/mL, from about 2 to about 5 U/mL, from about 5 to about 3,000 U/mL, from about 5 to about 2,500 U/mL, from about 5 to about 2,000 U/mL, from about 5 to about 1,500 U/mL, from about 5 to about 1,000 U/mL, from about 5 to about 500 U/mL, from about 5 to about 400 U/mL, from about 5 to about 300 U/mL, from about 5 to about 200 U/mL, from about 5 to about 100 U/mL, from about 5 to about 50 U/mL, from about 5 to about 20 U/mL, from about 5 to about 10 U/mL,

from about 10 to about 3,000 U/mL, from about 10 to about 2,500 U/mL, from about 10 to about 2,000 U/mL, from about 10 to about 1,500 U/mL, from about 10 to about 1,000 U/mL, from about 10 to about 500 U/mL, from about 10 to about 400 U/mL, from about 10 to about 300 U/mL, from about 10 to about 200 U/mL, from about 10 to about 100 U/mL, from about 10 to about 50 U/mL, from about 10 to about 20 U/mL, from about 10 to about 10 U/mL, from about 10 to about 5 U/mL, from about 10 to about 2 U/mL, from about 10 to about 1 U/mL, from about 10 to about 0.5 U/mL, from about 10 to about 0.1 U/mL, from about 20 to about 3,000 U/mL, from about 20 to about 2,500 U/mL, from about 20 to about 2,000 U/mL, from about 20 to about 1,500 U/mL, from about 20 to about 1,000 U/mL, from about 20 to about 500 U/mL, from about 20 to about 400 U/mL, from about 20 to about 300 U/mL, from about 20 to about 200 U/mL, from about 20 to about 100 U/mL, from about 20 to about 50 U/mL, from about 20 to about 20 U/mL, from about 20 to about 10 U/mL, from about 20 to about 5 U/mL, from about 20 to about 2 U/mL, from about 20 to about 1 U/mL, from about 20 to about 0.5 U/mL, from about 20 to about 0.1 U/mL, from about 50 to about 3,000 U/mL, from about 50 to about 2,500 U/mL, from about 50 to about 2,000 U/mL, from about 50 to about 1,500 U/mL, from about 50 to about 1,000 U/mL, from about 50 to about 500 U/mL, from about 50 to about 400 U/mL, from about 50 to about 300 U/mL, from about 50 to about 200 U/mL, from about 50 to about 100 U/mL, from about 50 to about 50 U/mL, from about 50 to about 20 U/mL, from about 50 to about 10 U/mL, from about 50 to about 5 U/mL, from about 50 to about 2 U/mL, from about 50 to about 1 U/mL, from about 50 to about 0.5 U/mL, from about 50 to about 0.1 U/mL, from about 100 to about 3,000 U/mL, from about 100 to about 2,500 U/mL, from about 100 to about 2,000 U/mL, from about 100 to about 1,500 U/mL, from about 100 to about 1,000 U/mL, from about 100 to about 500 U/mL, from about 100 to about 400 U/mL, from about 100 to about 300 U/mL, from about 100 to about 200 U/mL, from about 100 to about 100 U/mL, from about 100 to about 50 U/mL, from about 100 to about 20 U/mL, from about 100 to about 10 U/mL, from about 100 to about 5 U/mL, from about 100 to about 2 U/mL, from about 100 to about 1 U/mL, from about 100 to about 0.5 U/mL, from about 100 to about 0.1 U/mL, from about 200 to about 3,000 U/mL, from about 200 to about 2,500 U/mL, from about 200 to about 2,000 U/mL, from about 200 to about 1,500 U/mL, from about 200 to about 1,000 U/mL, from about 200 to about 500 U/mL, from about 200 to about 400 U/mL, from about 200 to about 300 U/mL, from about 200 to about 200 U/mL, from about 200 to about 100 U/mL, from about 200 to about 50 U/mL, from about 200 to about 20 U/mL, from about 200 to about 10 U/mL, from about 200 to about 5 U/mL, from about 200 to about 2 U/mL, from about 200 to about 1 U/mL, from about 200 to about 0.5 U/mL, from about 200 to about 0.1 U/mL, from about 300 to about 3,000 U/mL, from about 300 to about 2,500 U/mL, from about 300 to about 2,000 U/mL, from about 300 to about 1,500 U/mL, from about 300 to about 1,000 U/mL, from about 300 to about 500 U/mL, from about 300 to about 400 U/mL, from about 300 to about 300 U/mL, from about 300 to about 200 U/mL, from about 300 to about 100 U/mL, from about 300 to about 50 U/mL, from about 300 to about 20 U/mL, from about 300 to about 10 U/mL, from about 300 to about 5 U/mL, from about 300 to about 2 U/mL, from about 300 to about 1 U/mL, from about 300 to about 0.5 U/mL, from about 300 to about 0.1 U/mL, from about 400 to about 3,000 U/mL, from about 400 to about 2,500 U/mL, from about 400 to about 2,000 U/mL, from about 400 to about 1,500 U/mL, from about 400 to about 1,000 U/mL, from about 400 to about 500 U/mL, from about 400 to about 400 U/mL, from about 400 to about 300 U/mL, from about 400 to about 200 U/mL, from about 400 to about 100 U/mL, from about 400 to about 50 U/mL, from about 400 to about 20 U/mL, from about 400 to about 10 U/mL, from about 400 to about 5 U/mL, from about 400 to about 2 U/mL, from about 400 to about 1 U/mL, from about 400 to about 0.5 U/mL, from about 400 to about 0.1 U/mL, from about 500 to about 3,000 U/mL, from about 500 to about 2,500 U/mL, from about 500 to about 2,000 U/mL, from about 500 to about 1,500 U/mL, from about 500 to about 1,000 U/mL, from about 500 to about 500 U/mL, from about 500 to about 400 U/mL, from about 500 to about 300 U/mL, from about 500 to about 200 U/mL, from about 500 to about 100 U/mL, from about 500 to about 50 U/mL, from about 500 to about 20 U/mL, from about 500 to about 10 U/mL, from about 500 to about 5 U/mL, from about 500 to about 2 U/mL, from about 500 to about 1 U/mL, from about 500 to about 0.5 U/mL, from about 500 to about 0.1 U/mL, from about 1,000 to about 3,000 U/mL, from about 1,000 to about 2,500 U/mL, from about 1,000 to about 2,000 U/mL, from about 1,000 to about 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to about 5 U/mL, from about 2,000 to about 2 U/mL, from about 2,000 to about 1 U/mL, from about 2,000 to about 0.5 U/mL, from about 2,000 to about 0.1 U/mL, from about 2,500 to about 3,000 U/mL, from about 2,500 to about 2,500 U/mL, from about 2,500 to about 2,000 U/mL, from about 2,500 to about 1,500 U/mL, from about 2,500 to about 1,000 U/mL, from about 2,500 to about 500 U/mL, from about 2,500 to about 400 U/mL, from about 2,500 to about 300 U/mL, from about 2,500 to about 200 U/mL, from about 2,500 to about 100 U/mL, from about 2,500 to about 50 U/mL, from about 2,500 to about 20 U/mL, from about 2,500 to about 10 U/mL, from about 2,500 to about 5 U/mL, from about 2,500 to about 2 U/mL, from about 2,500 to about 1 U/mL, from about 2,500 to about 0.5 U/mL, from about 2,500 to about 0.1 U/mL, from about 3,000 to about 3,000 U/mL, from about 3,000 to about 2,500 U/mL, from about 3,000 to about 2,000 U/mL, from about 3,000 to about 1,500 U/mL, from about 3,000 to about 1,000 U/mL, from about 3,000 to about 500 U/mL, from about 3,000 to about 400 U/mL, from about 3,000 to about 300 U/mL, from about 3,000 to about 200 U/mL, from about 3,000 to about 100 U/mL, from about 3,000 to about 50 U/mL, from about 3,000 to about 20 U/mL, from about 3,000 to about 10 U/mL, from about 3,000 to about 5 U/mL, from about 3,000 to about 2 U/mL, from about 3,000 to about 1 U/mL, from about 3,000 to about 0.5 U/mL, from about 3,000 to about 0.1 U/mL, or from about 2,500 to about 3,000 U/mL of proclotting enzyme (e.g., recombinant proclotting enzyme). Proclotting enzyme enzymatic activity may be assayed by any method known in the art, including, for example, by measuring cleavage of the chromogenic substrate Ac-Ilu-Glu-Gly-Arg-pNA, for example, as described in Example 1 hereinbelow. As used herein one unit (U) of proclotting enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 micromole of a substrate (e.g., Ac-Ilu-Glu-Gly-Arg-pNA) per minute.

E. Factor C

[0076] As used herein, the term “factor C” refers to a zymogen, or a functional fragment thereof, that is capable of being activated by endotoxin, and is capable of cleaving (e.g., enzymatically cleaving) factor B to form an activated

factor B. The term factor C includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type factor C sequence, and/or fusion proteins or conjugates including a factor C. As used herein, the term “functional fragment” of a factor C refers to fragment of a full-length factor C that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring factor C. Factor C enzymatic activity may be assayed by any method known in the art, including, for example, by measuring cleavage of the chromogenic substrate Z-Val-Pro-Arg-pNA, for example, as described in Example 1 herein. In certain embodiments, the functional fragment comprises at least 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 consecutive amino acids present in a full-length, naturally occurring factor C.

[0077] It is contemplated that any horseshoe crab factor C, for example, a *Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas*, or *Carcinoscorpius rotundicauda* factor C, may be used in the practice of the invention.

[0078] A DNA sequence encoding an exemplary *Limulus polyphemus* factor C is depicted in SEQ ID NO: 1. Exemplary *Limulus polyphemus* factor C amino acid sequences are depicted in SEQ ID NOs: 2 and 3. SEQ ID NO: 2 is the mature form whereas SEQ ID NO:3 includes the signal sequence as residues 1-25. A DNA sequence encoding an exemplary *Tachypleus tridentatus* factor C is depicted in SEQ ID NO: 10. Exemplary *Tachypleus tridentatus* factor C amino acid sequences are depicted in SEQ ID NOs: 11 and 12. SEQ ID NO: 11 is the mature form whereas SEQ ID NO: 12 includes the signal sequence as residues 1-21. In certain embodiments, a factor C comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 11, or SEQ ID NO: 12, or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, at least 99.5%, or at least 99.8% to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 11, or SEQ ID NO: 12. In certain embodiments, the factor C comprises a conservative substitution relative to a wild-type factor C sequence or a factor C sequence disclosed herein.

[0079] In certain embodiments, the factor C is a recombinant factor C. In certain embodiments, the factor C is a native factor C, e.g., an isolated native factor C.

[0080] In certain embodiments, a recombinant horseshoe crab factor C for use as an endotoxin detection reagent, either alone, or in combination with other horseshoe crab proteins (e.g., factor B, proclotting enzyme and/or factor C, whether sourced naturally or produced recombinantly) is expressed in a mammalian cell, for example, a Chinese hamster ovary (CHO) or human embryonic kidney (HEK) cell. In certain embodiments, the recombinant factor C is glycosylated differently (e.g., has one or more different glycosyl groups or a glycosylation pattern) when compared to factor C present in the native horseshoe crab amebocyte lysate, or when compared to factor C produced recombinantly in distinct expression host cells. As a non-limiting example, factor C may be produced recombinantly in a gene-edited cell line, such as GnTI⁻ HEK cells (e.g., HEK cells that do not have N-acetylglucosaminyltransferase I (GnTI) activity) that produce factor C proteins lacking (α -2,3)-linked terminal sialic acid. Use of such readily

available gene-edited cell lines may be desirable for high-yield expression of homogeneously glycosylated recombinant proteins.

F. Methods of Making Recombinant Proteins

[0081] Methods for producing recombinant proteins known in the art. For example, DNA molecules encoding a protein of interest (e.g., factor B, proclotting enzyme and/or factor C, as disclosed herein) can be synthesized chemically or by recombinant DNA methodologies. The resulting DNA molecules encoding the protein of interest can be ligated to other appropriate nucleotide sequences, including, for example, expression control sequences, to produce conventional gene expression constructs (i.e., expression vectors) encoding the desired protein. Production of defined gene constructs is within routine skill in the art.

[0082] Nucleic acids encoding desired proteins (e.g., factor B, proclotting enzyme and/or factor C, as disclosed herein) can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the protein of interest.

[0083] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, e.g., Trp or Tac, and a prokaryotic signal sequence. The expressed protein may be secreted. The expressed protein may accumulate in refractile or inclusion bodies, which can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the protein may be refolded and/or cleaved by methods known in the art.

[0084] If the engineered gene is to be expressed in eukaryotic host cells, e.g., CHO or HEK cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, a poly A sequence, and a stop codon. Optionally, the vector or gene construct may contain enhancers and introns. The gene construct can be introduced into eukaryotic host cells using conventional techniques.

[0085] A polypeptide or protein of interest (e.g., factor B, proclotting enzyme and/or factor C, as disclosed herein) can be produced by growing (culturing) a host cell transfected with an expression vector encoding such a polypeptide or protein, under conditions that permit expression of the polypeptide or protein. Following expression, the polypeptide can be harvested and purified or isolated using techniques known in the art, e.g., affinity tags such as glutathione-S-transferase (GST) or histidine tags.

[0086] Provided herein are isolated nucleic acids comprising the sequence of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 13, or SEQ ID NO: 16. These sequences can be spliced into a suitable expression vector and operatively linked to a promoter for use in a desired expression host using standard recombinant DNA methodologies. Also provided are expression host cells (e.g., mammalian host cells) comprising such an expression vec-

tor, which can then be used to express the protein encoded by one or more of the foregoing nucleic acid sequences. Exemplary methods for making recombinant factor B, proclotting enzyme and factor C are described in Example 1 herein.

[0087] In one embodiment, provided herein is a method of producing a horseshoe crab recombinant factor C protein, the method comprising: (a) expressing a nucleic acid sequence encoding the recombinant horseshoe crab factor C in a host cell (e.g., a HEK293 cell or a CHO cell) engineered to remove a glycosyltransferase enzyme (e.g., N-acetylglucosaminyltransferase); and (b) purifying the recombinant factor C expressed in the host cell. Also provided is a recombinant horseshoe crab factor C protein (e.g., a factor C protein that does not contain (α -2,3)-linked terminal sialic acid) produced by such a method. The resulting recombinant horseshoe crab factor C can be used to prepare an endotoxin detection reagent (e.g., a hybrid lysate), by admixing such a recombinant factor C with a composition comprising recombinant horseshoe crab factor B and recombinant horseshoe crab proclotting enzyme.

[0088] It is understood that a recombinantly expressed polypeptide or protein may have a different molecular weight and/or be differently glycosylated relative to a corresponding native polypeptide or protein. Similarly, a protein or polypeptide recombinantly expressed in a first host cell type may have a different molecular weight and/or be differently glycosylated relative to a corresponding protein or polypeptide expressed in a second, different host cell type. Glycosylation of recombinant proteins produced in mammalian host cells is described, for example, in Lis et al. (1993) EUR. J. BIOCHEM. 218:1-27, Parodi (2000) ANNU. REV. BIOCHEM. 69:69-93, Viswanathan et al. (2005) BIOCHEM. 44:7526-7534, Tomiya et al. (2004) GLYCOCONJUGATE JOURNAL 21: 343-360, Tomiya et al. (2003) ACC. CHEM. RES. 36:613-620, Gerngross (2004) NAT. BIOTECHNOL. 22:1409-1414, and Demain et al. (2009) BIOTECHNOLOGY ADVANCES 27:297-306.

G. Methods of Making Hybrid Lysate

[0089] Hybrid amebocyte lysates may be produced by combining native and recombinant components disclosed herein.

[0090] The invention provides a method for preparing a microbial contaminant (e.g., endotoxin) detection reagent, for increasing the sensitivity of a native horseshoe crab amebocyte lysate for a microbial contaminant (e.g., endotoxin), and/or reducing the amount of a native horseshoe crab amebocyte lysate required to detect a microbial contaminant (e.g., endotoxin). The method comprises adding a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme to a native horseshoe crab amebocyte lysate. In certain embodiments, the method comprises diluting the native horseshoe crab amebocyte lysate. For example, a contemplated method comprises diluting a native horseshoe crab amebocyte lysate to produce a diluted horseshoe crab amebocyte lysate; and adding to the diluted native horseshoe crab amebocyte lysate a recombinant horseshoe crab factor B and/or a recombinant horseshoe crab proclotting enzyme. In certain embodiments, the native horseshoe crab amebocyte lysate is diluted by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1,000%.

[0091] Hybrid amebocyte lysate compositions preferably are sterile. Sterilization can be accomplished by any suitable method, e.g., filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[0092] Hybrid amebocyte lysate compositions can also be dried onto a solid surface (such as a vial, a cartridge or a 12-well or 96-well plate), such as by lyophilization. Prior to drying, one or more additives are optionally admixed with the hybrid amebocyte lysate. For example, a resolubilizing and/or an anti-flaking agent can be included. The resolubilizing agent is an agent that, either alone or in combination with another resolubilizing agent, facilitates the resolubilization of one or more components of the hybrid amebocyte lysate once the hybrid amebocyte lysate is exposed to a fluid sample. The resolubilizing agent preferably also stabilizes the lysate in its dried form. The resolubilizing agent provided in the mixture facilitates the stability of the reagents and their dissolution during the assay. Resolubilizing agents include, for example, one or more sugars, salts, or combinations thereof. Preferred sugar resolubilizing agents include, for example, mannitol, mannose, sorbitol, trehalose, maltose, dextrose, sucrose, and other monosaccharides or disaccharides. The anti-flaking agent included in the mixture further stabilizes the reagents and reduces flaking of the dried lysate. The anti-flaking agent preferably also stabilizes the lysate in its dried form. Preferred anti-flaking agents include, for example, one or more polymers, for example, polyethylene glycol, polyvinyl pyrrolidone, polyvinyl alcohol, mannitol, dextran, and proteins, for example, serum albumin. An anti-frothing agent such as polyvinyl alcohol or polypropylene glycol can also be included. Salts and/or buffers, such as sodium chloride, magnesium sulfate, and HEPES buffer can also be included. Other kinds of buffers, such as TRIS-HCl buffer, TES, MOPS, PIPES, BES, MOPSO, DIPSO, MOBS, TAPSO, HEPPSO, POPSO, TEA, EPPS, Tricine, and phosphate can be used, as can other buffers with buffering capacity between pH 7 and pH 8, as this is a preferred range of pH for the reaction. The target pH of the composition, after admixture with a sample, is preferably between 7.3 and 8.0.

[0093] The mixture can be dried onto a surface of the conduit in an environment having a temperature of about 4° C. to about 40° C., more preferably, from about 10° C. to about 35° C., more preferably, from about 15° C. to about 30° C. and a relative humidity of about 0% to about 30%, more preferably, from about 2% to about 20%, more preferably, from about 4% to about 10%. Preferably, the temperature is about 25° C. and the relative humidity is about 5%. Drying preferably occurs for about 10 minutes to about 8 hours, more preferably for about 1 hour in a temperature regulated drying chamber.

[0094] In another embodiment, the mixture is dried onto the surface of the conduit by lyophilization or freeze-drying, for example, at temperatures below 0° C., for example, from about -75° C. to about -10° C., more preferably from about -30° C. to about -20° C.

II. Methods of Detection and Sample Preparation Considerations

[0095] Hybrid amebocyte lysates disclosed herein can be used in various assays to detect a microbial contaminant (e.g., endotoxin). The invention provides a method of detect-

ing the presence and/or amount of a microbial contaminant (e.g., endotoxin) in a sample. The method comprises contacting a hybrid amebocyte lysate (e.g., a hybrid amebocyte lysate disclosed herein) with a sample (e.g., a sample suspected of containing endotoxin), allowing the hybrid amebocyte lysate to react with the sample to produce a detectable product (e.g., a gel, increase in turbidity, or a colored or light-emitting product), and detecting the detectable product (e.g., visually or by the use of an optical detector).

[0096] Hybrid amebocyte lysates disclosed herein can be used to detect a microbial contaminant (e.g., endotoxin) using, for example, endpoint or kinetic assays. Exemplary endpoint assays include an endpoint chromogenic assay or an endpoint turbidimetric assay. Exemplary kinetic assays include a kinetic turbidimetric assay, a one-step kinetic assay or a multi-step kinetic assay. Each of the assays is discussed in more detail below. Furthermore, it is understood that the assays may be modified to be performed in a particular assay format, for example, in a cartridge or in the well of a plate, for example, a 96 well plate.

A. Kinetic Assays

[0097] Exemplary kinetic assays include multi-step kinetic assays, single-step kinetic assays, and kinetic turbidimetric assays.

[0098] (i) Multi-Step Kinetic Assay

[0099] A multi-step kinetic assay (for example, as described in U.S. Pat. No. 7,329,538) is initiated by combining the sample to be tested with a volume of hybrid amebocyte lysate to produce a sample-hybrid amebocyte lysate mixture. The mixture then is incubated for a predetermined period of time. The mixture then is contacted with a substrate, for example, a chromogenic or fluorogenic substrate, to produce a sample-hybrid amebocyte lysate-substrate mixture. Thereafter, the time in which a preselected change in an optical property (for example, a specific change in an absorbance value or a specific change in a transmission value) is measured.

[0100] The assay can be calibrated by measuring the time in which a preselected change in an optical property occurs when a certain amount of a microbial contaminant (e.g., endotoxin) is introduced into the assay. By comparing the result generated by a test sample against the results generated by one or more known amounts of the microbial contaminant (e.g., endotoxin), it is possible to detect the presence or amount of the microbial contaminant (e.g., endotoxin) in a test sample.

[0101] It is understood that a multi-step kinetic assay can be run in a cartridge format. The cartridge preferably is used with an optical detector, for example, a hand-held optical detector as shown and described in U.S. Pat. No. Des. 390,661.

[0102] By way of example and as illustrated in FIGS. 8A-8D, cartridge 1 has a substantially planar housing fabricated, for example, from a moldable biocompatible material. The housing may be fabricated from any material, however, transparent and/or translucent glass or polymers are preferred. Preferred polymers include, for example, polystyrene, polycarbonate, acrylic, polyester, optical grade polymers, or any plastic such that the optical cell is substantially transparent. The housing contains at least one fluid inlet port 4, at least one optical cell 6, and at least one conduit 8 having a fluid contacting surface for providing

fluid flow communication between the fluid inlet port 4 and optical cell 6. The only requirements for the optical cell 6 are that it defines a void capable of containing a sample to be tested and that a portion of the optical cell 6 is transparent to light. Cartridge 1 may also have at least one pump port 12 in fluid flow communication with fluid inlet port 4 and optical cell 6 for attaching the cartridge 1 to a pump. The pump may then impart a negative pressure via pump port 12 to pull the sample from fluid inlet port 4 to optical cell 6. A hybrid amebocyte lysate is disposed on a first region 14 of the fluid contacting surface of conduit 8, so that when a sample is applied to fluid inlet port 4, the sample traverses region 14 and solubilizes or reconstitutes the hybrid amebocyte lysate into the sample as it moves toward optical cell 6.

[0103] A second region 16 of the fluid contacting surface of conduit 8 is spaced apart from and downstream of first region 14. In this configuration, hybrid amebocyte lysate is disposed at first region 14 and a chromogenic or fluorogenic substrate is disposed at second region 16, so that after the sample is contacted with the hybrid amebocyte lysate in region 14, the sample-lysate mixture traverses conduit 8 and contacts the substrate in region 16. The sample-lysate-substrate mixture then traverses conduit 8 to optical cell 6.

[0104] The cartridges can be designed and used according to the type and/or number of tests required. For example, a single sample may be tested, for example, in duplicate or triplicate, for example, for research laboratory use or for medical device and biopharmaceutical testing. Alternatively, two or more different samples may be tested individually. The cartridge preferably is a single-use, disposable cartridge that is discarded after one use. The cartridges typically use approximately 20-100 fold less hemocyte lysate per sample than is used in the conventional endpoint chromogenic or kinetic chromogenic assays performed in multi-well plates, and thus provides a less costly and environmentally-friendlier test.

[0105] With reference to FIG. 8A, in order to perform a multi-step kinetic assay in an exemplary cartridge 1, a sample is first moved, for example, by pump action, to a first region 14 containing the hybrid amebocyte lysate, where it is mixed and incubated for a predetermined period of time. The sample-hybrid amebocyte lysate mixture then is moved, for example, by pump action, to the second region 16 containing the substrate, for example, a chromogenic or fluorogenic substrate, where it is solubilized. The sample-substrate mixture then is moved to optical cell 6, for a measurement of an optical property. The time intervals required for mixing and incubating steps are preprogrammed for optimal specificity and sensitivity to the microbial contaminant (e.g., endotoxin) concentration range of interest.

[0106] Although the multi-step assay may be performed in a cartridge of the type discussed above, it may also be employed in a variety of other formats, for example, within the well of a microtiter plate. In this type of assay, a sample of interest is combined with a hybrid amebocyte lysate and incubated for a predetermined period of time. Then, after the predetermined period of time, a chromogenic or fluorogenic substrate is added to the well. After mixing, the time in which a preselected change in an optical property occurs is measured. The result can then be compared against one or more standard values to measure the presence or amount of a microbial contaminant (e.g., endotoxin) in the sample.

[0107] In the well-type format, the samples and reagents are added to each of the wells, preferably using an automated system, such as a robot, and the plate processed by a microplate reader, which can be programmed to sequentially read the absorbance of each well in a repetitive fashion.

[0108] (ii) Single-Step Kinetic Assay

[0109] A single-step kinetic assay, for example, a single step-chromogenic assay, is described in U.S. Pat. No. 5,310, 657. Briefly, a kinetic chromogenic assay includes the steps of (i) simultaneously solubilizing a hybrid amebocyte lysate with a sample to be analyzed and a substrate, for example, a chromogenic substrate, (ii) incubating the resulting mixture at a temperature of about 0° to about 40° C., preferably about 25° to about 40° C., over a predetermined time range and (iii) measuring a time required for a calorimetric change to reach a pre-selected value or change of the calorimetric readout, using a conventional spectrophotometer.

[0110] This type of assay, like the multi-step kinetic assay, can be performed in a cartridge or a well-type format. A cartridge similar to that described above for the multi-step kinetic assay can be modified for use in single-step kinetic assay. With reference to FIG. 8A, chromogenic or fluorogenic substrate is applied, for example, to the surface of conduit 8 at first region 14 together with the hybrid amebocyte lysate. To perform a kinetic assay in cartridge 1 and in reference to FIG. 8A, a sample is moved, for example, by pump action, to a first region 14 of the conduit 8 containing both the hybrid amebocyte lysate and substrate, where they are solubilized, for example, by cycling between forward and reverse pump action. The sample-hybrid amebocyte lysate-substrate mixture then is moved to optical cell 6 for measurement of an optical property, for example, the absorbance or transmittance properties of the sample by an optical detector. The detector may determine how long it takes for each optical property to exhibit, for example, a 5% drop in optical transmittance. Results from multiple assays, for example, two assays, can be averaged.

[0111] The assay can be calibrated by measuring the time in which a preselected change in an optical property occurs when a certain amount of a microbial contaminant (e.g., endotoxin) is introduced into the assay. By comparing the result generated by a test sample against one or more results with known amounts of the microbial contaminant (e.g., endotoxin), it is possible to measure the presence or amount of the microbial contaminant (e.g., endotoxin) in the test sample.

[0112] This type of assay format may be employed in a variety of other formats, for example, within the well of a microtiter plate. In this type of assay, a sample of interest is mixed with a hybrid amebocyte lysate and a chromogenic or fluorogenic substrate. After mixing, the time in which a preselected change in an optical property occurs is measured. The result can then be compared against standard values to measure the presence or amount of a microbial contaminant (e.g., endotoxin) in the sample of interest.

[0113] (iii) Kinetic Turbidimetric Assay

[0114] A kinetic turbidimetric assay can include the steps of (i) solubilizing a hybrid amebocyte lysate with a sample to be analyzed, (ii) incubating the resulting mixture at a temperature of about 0° to about 40° C., preferably about 25° to about 40° C., over a predetermined time range, and (iii) measuring a time required for either a turbidity change caused by coagulation to reach a pre-selected value or a ratio

in change of the turbidity, using a conventional coagulometer, nephrometer, or spectrophotometer.

[0115] This type of assay, like the previous assays, can be performed in a cartridge or a well-type format. A cartridge similar to that described above for the multi-step or single-step kinetic assays can be modified for use in kinetic turbidimetric assays. With reference to FIG. 8A, no chromogenic or fluorogenic substrate needs to be applied to either first region 14 or second region 16.

[0116] Referring to FIG. 8A, in order to perform a kinetic turbidimetric assay in a cartridge 1, a sample is, for example, moved to a first region 14 of the conduit 8 containing the hybrid amebocyte lysate, where it is solubilized, for example, by cycling between forward and reverse pump action. The sample-lysate mixture then is moved to optical cell 6 for measurement of an optical property, for example, turbidity, by measuring, for example, the absorbance or transmittance properties of the sample-lysate mixture using an optical detector. The detector may determine how long it takes for each optical property to exhibit, for example, a 5% drop in optical transmittance. Results from multiple assays, for example, two assays can be averaged.

[0117] The assay can be calibrated by measuring the time in which a preselected change in an optical property, for example, turbidity, occurs when a certain amount of a microbial contaminant (e.g., endotoxin) is introduced into the assay. By comparing the result generated by a test sample against one or more results with known amounts of the microbial contaminant (e.g., endotoxin), it is possible to measure the presence or amount of the microbial contaminant (e.g., endotoxin) in the test sample.

[0118] This type of assay format may be employed in a variety of other formats, for example, within the well of a microtiter plate. In this type of assay, a sample of interest is mixed with a hybrid amebocyte lysate. After mixing, the time in which a preselected change in an optical property, for example, turbidity, occurs is measured. The result can then be compared against standard values to measure the presence or amount of a microbial contaminant (e.g., endotoxin) in the sample of interest.

B. Endpoint Assays

[0119] Exemplary endpoint assays include endpoint chromogenic or fluorogenic and endpoint turbidimetric assays.

[0120] (i) Endpoint Chromogenic or Fluorogenic Assay

[0121] Endpoint chromogenic or fluorogenic assays can include the steps of (i) solubilizing a hybrid amebocyte lysate with a sample to be analyzed, (ii) incubating the resulting mixture at a temperature of about 0° to about 40° C., preferably about 25° to about 40° C., for a predetermined time, (iii) contacting substrate, for example, a chromogenic or fluorogenic substrate, with the incubated sample-hybrid amebocyte lysate mixture, (iv) optionally adding a reaction inhibitor, for example, acetic acid, and (v) measuring, for example by calorimetric change, a substance produced from the substrate by enzymatic activity.

[0122] This type of assay can be performed in a cartridge or in a well-type format. When an endpoint chromogenic or fluorogenic assay is performed in a cartridge 1 (see, FIG. 8A), a sample is moved, for example, to a first region 14 of the conduit 8 containing the hybrid amebocyte lysate, where it is solubilized, for example, by cycling between forward and reverse pump action. Following a predetermined incubation period, the sample-hybrid amebocyte lysate mixture

then is moved, for example, by pump action to a second region **16** of the conduit **8** containing the chromogenic or fluorogenic substrate, where it is solubilized, for example, by cycling between forward and reverse pump action. The sample-hybrid amebocyte lysate-substrate mixture optionally then is moved to a third region containing a reaction inhibitor. Afterwards, the sample-hybrid amebocyte lysate-substrate mixture is moved to optical cell **6** for measurement of an optical property, for example, the absorbance or transmittance properties of the sample by an optical detector. It is contemplated, however, that when performing an endpoint chromogenic or fluorogenic assay in a cartridge it is not necessary to stop the reaction using a reaction inhibitor. Under this type of assay, the final optical readings (endpoint readings) are recorded at a predetermined time.

[0123] The assay can be calibrated by measuring an optical property, for example, absorbance or transmittance, when a certain amount of a microbial contaminant (e.g., endotoxin) is introduced into the assay. By comparing the result generated by a test sample against one or more results with known amounts of the microbial contaminant (e.g., endotoxin), it is possible to measure the presence or amount of the microbial contaminant (e.g., endotoxin) in the test sample.

[0124] As discussed, this type of assay format may be employed in a variety of other formats, for example, within the well of a microtiter plate. In this type of assay, a sample of interest is mixed with a hybrid amebocyte lysate and incubated for a preselected period of time. Then, a chromogenic or fluorogenic substrate is added to the mixture and the sample incubated for another period of time. Then a reaction inhibitor, for example, acetic acid, is added to the sample, and an optical property of the sample, for example, absorbance or transmittance, is measured. The result can then be compared against standard values to measure the presence or amount of a microbial contaminant (e.g., endotoxin) in the sample of interest.

[0125] (ii) Endpoint Turbidimetric Assay

[0126] End point turbidimetric assays can include the steps of (i) solubilizing a hybrid amebocyte lysate with a sample to be analyzed, (ii) incubating the resulting mixture at a temperature of about 0° to about 40° C., preferably about 25° to about 40° C., for a predetermined time, (iii) optionally adding a reaction inhibitor, for example, acetic acid, and (iv) measuring the increase in turbidity as a result of coagulation, if any, using a conventional coagulometer, nephrometer, or spectrophotometer.

[0127] Endpoint turbidimetric assays can be performed in a cartridge-type format. With reference to FIG. **8A**, a sample is applied to cartridge **1** and is moved, for example, to a first region **14** of the conduit **8** containing the hemocyte lysate, where it is solubilized, for example, by cycling between forward and reverse pump action. The sample-lysate mixture then is moved to optical cell **6** for measurement of an optical property, for example, turbidity, using an optical detector. Results from multiple assays, for example, two assays can be averaged.

[0128] The assay can be calibrated, for example, by measuring the turbidity at a preselected time when a certain amount of a microbial contaminant (e.g., endotoxin) is introduced into the assay. By comparing the result generated by a test sample against one or more results with known amounts of the microbial contaminant (e.g., endotoxin), it is

possible to measure the presence or amount of the microbial contaminant (e.g., endotoxin) in the test sample.

[0129] This type of assay format may also be run in other formats, for example, within the well of a microtiter plate. In this type of assay, a sample of interest is mixed with a hybrid amebocyte lysate and incubated for a preselected period of time. The reaction can then be stopped by the addition of an inhibitor. An optical property, for example, turbidity, of the sample then is measured at a preselected time point. The result can then be compared against standard values to measure the presence or amount of the microbial contaminant (e.g., endotoxin) in the sample of interest.

C. Specimen Collection and Preparation Considerations

[0130] In general, materials used to harvest, store, or otherwise contact a sample to be tested, as well as test reagents, should be free of microbial contamination, for example, should be pyrogen-free. Materials may be rendered pyrogen-free by, for example, heating at 250° C. for 30 minutes. Appropriate precautions should be taken to protect depyrogenated materials from subsequent environmental contamination.

[0131] The hybrid amebocyte lysate may be used to measure the presence or amount of a microbial contaminant (e.g., endotoxin) in a sample of interest, for example, in a fluid, for example, a fluid to be administered locally or systemically, for example, parenterally to a mammal, or a body fluid to be tested for infection, including, for example, blood, lymph, urine, serum, plasma, ascites fluid, lung aspirants, and the like. In addition, the assays may be used to detect a microbial contaminant (e.g., endotoxin) present on a surface. For example, the surface of interest is swabbed and the swab then is introduced into or dissolved in liquid. The liquid can then be assayed as described herein.

[0132] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[0133] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[0134] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the

present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

[0135] The articles “a” and “an” are used in this disclosure to refer to one or more than one (i.e., to at least one) of the grammatical object of the article, unless the context is inappropriate. By way of example, “an element” means one element or more than one element.

[0136] The term “and/or” is used in this disclosure to mean either “and” or “or” unless indicated otherwise.

[0137] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[0138] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[0139] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred from the context.

[0140] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0141] At various places in the present specification, variable or parameters are disclosed in groups or in ranges. It is specifically intended that the description include each and every individual subcombination of the members of such groups and ranges. For example, an integer in the range of 0 to 40 is specifically intended to individually disclose 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and an integer in the range of 1 to 20 is specifically intended to individually disclose 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

[0142] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

[0143] As a general matter, compositions specifying a percentage are by weight unless otherwise specified.

[0144] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The abbreviations used herein have their conventional meaning within the chemical and biological arts.

EXAMPLES

[0145] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1

[0146] This Example describes the preparation of recombinant *Limulus polyphemus* factor C, factor B, and pro-clotting enzyme.

[0147] DNA sequences encoding *Limulus polyphemus* factor C, factor B and pro-clotting enzyme (with codon usage optimized for expression in mammalian cells) were cloned into expression plasmid BD609 (ATUM). The DNA sequence encoding *Limulus polyphemus* factor C is depicted in SEQ ID NO: 1 (and the corresponding amino acid sequence is depicted in SEQ ID NO: 3). The DNA sequence encoding *Limulus polyphemus* factor B is depicted in SEQ ID NO: 4 (and the corresponding amino acid sequence is depicted in SEQ ID NO: 6). The DNA sequence encoding *Limulus polyphemus* pro-clotting enzyme is depicted in SEQ ID NO: 7 (and the corresponding amino acid sequence is depicted in SEQ ID NO: 9). Expression plasmids were transfected into HEK-293 cells using the FreeStyle 293 Expression System (Thermo Fisher) to generate a stable clonal cell lines.

[0148] For expression and purification, HEK-293 cells were thawed and added to FreeStyle 293 Expression Media in a flask. Cells were grown at 37° C., 5-7% CO₂ at 120 rpm and passaged every 24-72 hours. When cells reached the desired volume and density, they were used to seed a total of 20 L of culture in a WAVE Bioreactor. After 72 hours, the supernatant was harvested by centrifugation at 4,000×g for 15 minutes followed by sterile filtration. The supernatant was concentrated to <2 L and buffer exchanged by Tangential Flow Filtration (TFF, GE Life Sciences). The TFF system was equilibrated with 20 mM Tris-HCl buffer pH 8.0 containing 20 mM NaCl.

[0149] To mitigate endotoxin exposure, all materials used were single use. Water for injection was used for all buffers, and all buffers were made on the day of use.

[0150] The amount of each recombinant protein was measured in terms of activity (U/mL) assayed under the following conditions. Recombinant factor C (rFC) was activated by addition of endotoxin (1 µg/mL lipopolysaccharide from *E. coli* O55:B5 (List Labs)) and incubation at 37° C. for 30 minutes. Then, the cleavage of the synthetic substrate Z-Val-Pro-Arg-pNA was measured by monitoring absorbance at 405 nm at 37° C. Recombinant factor B (rFB) was activated by addition of activated rFC and incubation at 37° C. for 1 hour. Then, the cleavage of the synthetic substrate H-D-Leu-Thr-Arg-pNA was measured by monitoring absorbance at 405 nm at 37° C. Recombinant pro-clotting enzyme (rPCE) was activated by addition of activated rFB and incubation at 37° C. for 1 hour. Then, the cleavage of the synthetic substrate Ac-Ilu-Glu-Gly-Arg-pNA was measured by monitoring absorbance at 405 nm at 37° C. One unit of activity was defined as the amount of enzyme that catalyzed the conversion of 1 micromole of substrate per minute.

Example 2

[0151] This example describes the addition of recombinant *Limulus polyphemus* factor C, factor B, and/or pro-

clotting enzyme to crude *Limulus* amebocyte lysate (LAL), and the resulting endotoxin detection activity.

[0152] Crude LAL was prepared generally as described in Levin et al. (1968) THROMB. DIATH. HAEMORRH. 19:186. Briefly, hemolymph was harvesting from horseshoe crab. The resulting hemolymph was centrifuged to produce an amebocyte pellet. The amebocytes were then re-harvested, re-rinsed, and re-centrifuged. After second rinsing and harvesting steps, the resulting amebocytes were lysed by osmotic shock, and the resulting crude amebocyte lysate was stored at 2-8° C. until further use.

[0153] Recombinant *Limulus polyphemus* factor C (rFC), factor B (rFB), and pro-clotting enzyme (rPCE) were prepared as described in Example 1. A typical LAL endotoxin detection reagent includes 20% crude LAL, appropriate/compatible divalent cations, detergents, excipients for lyophilization, and substrate (Ac-Ilu-Glu-Gly-Arg-pNA). LAL endotoxin detection reagents were generated including reduced amounts of crude LAL (e.g., 10% or 5%), as well as additional recombinant proteins, as shown in TABLE 1.

TABLE 1

Reagent No.	Final concentration			
	Crude LAL (%)	rFC (U/mL)	rFB (U/mL)	rPCE (U/mL)
0	10	None	None	None
1	10	21.4	None	None
2	10	None	0.2	None
3	10	None	0.11	114
4	5	None	0.11	114
5	5	10.7	0.11	114
6	5	None	0.12	190

[0154] Endotoxin was detected using a kinetic chromogenic assay method (KCA). Briefly, 0.1 mL of LAL reagent was mixed with 0.1 mL of a sample containing RSE (US Reference Standard Endotoxin). Reaction of endotoxin with the LAL reagent leads to cleavage of the Ac-Ilu-Glu-Gly-Arg-pNA substrate in the LAL reagent and a resulting chromogenic signal that can be measured by absorbance at 405 nm. The activity of the LAL reagent was measured by onset time (the amount of time to reach to reach a particular absorbance (OD 0.05) at 405 nm. Onset time was measured with 0.02-20 EU/mL RSE, and logarithmic plotting of the onset times at each concentration was used to generate a standard curve.

[0155] The onset time at 0.2 EU/mL of RSE was compared for different reagents. Results are shown in FIG. 2. Activity in FIG. 2 is relative to an LAL reagent containing undiluted (20%) crude LAL, i.e., 100% activity in FIG. 2 corresponds to the activity (onset time for 0.2 EU/mL of RSE, calculated as described above) of an LAL reagent containing undiluted (20%) crude LAL.

[0156] Reagent No. 0 (including diluted (10%) crude LAL, with no additional recombinant proteins) showed 25% activity relative to a reagent including undiluted (20%) crude LAL. Addition of rFC alone (Reagent No. 1) did not increase relative activity. Addition of rFB alone (Reagent No. 2) did increase relative activity. However, the greatest increase in relative activity was observed following the addition of both rFB and rPCE. For certain reagents (Reagent Nos. 4-6) the relative activity was greater than 100%. In other words, even though these reagents contained diluted

crude LAL, they showed greater endotoxin detection activity than a corresponding reagent with undiluted crude LAL.

[0157] Together, these results show that the addition of rFB and/or rPCE to a native amebocyte lysate (e.g., native LAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KCA assay) while maintaining, or in certain instances even increasing, sensitivity of the amebocyte lysate.

Example 3

[0158] This example describes the addition of varying amounts of recombinant *Limulus polyphemus* factor B to crude *Limulus* amebocyte lysate (LAL), and the resulting impact on endotoxin detection activity.

[0159] Recombinant *Limulus polyphemus* factor B (rFB) and pro-clotting enzyme (rPCE) were prepared as described in Example 1, crude LAL was prepared as described in Example 2, and LAL reagent activity was assayed generally as described in Example 2.

[0160] LAL reagents were generated containing diluted crude LAL, rPCE, and varying amounts of rFB, as shown in TABLE 2. The rFB amounts used were 0, 0.2, and 0.4 U/mL.

[0161] Results are shown in FIG. 3 and TABLE 3. FIG. 3 depicts activity for the indicated reagents relative to the activity of Reagent No. 1 (an LAL reagent containing undiluted (20%) crude LAL and no recombinant proteins) at the same endotoxin concentration, i.e., 100% activity in FIG. 3 for a given endotoxin concentration corresponds to the activity of Reagent No. 1 at the same endotoxin concentration. TABLE 3 depicts onset times at the indicated endotoxin concentration for each reagent.

[0162] As shown in FIG. 3 and TABLE 3, addition of rPCE alone increased activity, as Reagent No. 2 (with diluted crude LAL and rPCE) had comparable activity to Reagent No. 1 (with undiluted crude LAL and no rPCE). However, the greatest increase in activity was observed following the addition of both rFB and rPCE, as seen in Reagent Nos. 3 and 4, which had substantially increased activity relative to Reagent No. 1. The increase in activity was generally in proportion to the amount of rFB that was added.

TABLE 2

Reagent No.	1	2	3	4
LAL	100% (20%)	50% (10%)	50% (10%)	50% (10%)
rPCE (U/mL)	None	1320	1320	1320
rFB (U/mL)	None	None	0.2	0.4

TABLE 3

Endotoxin (EU/mL)	Onset Time (Sec)			
	1	2	3	4
NC	5999	5678	5220	5820
0.01	4650	4035	3233	2820
0.1	2543	2370	1905	1650
1	1403	1373	1103	985
10	785	784	630	584

[0163] Together, these results show that the addition of rFB and/or rPCE to a native amebocyte lysate (e.g., native LAL) can reduce the amount of native amebocyte lysate

required to detect endotoxin (e.g., in a KCA assay) while maintaining, or in certain instances even increasing, sensitivity of the amebocyte lysate.

Example 4

[0164] This example describes the preparation of lyophilized LAL reagents containing diluted crude LAL and recombinant *Limulus polyphemus* factor B (rFB) and pro-clotting enzyme (rPCE).

[0165] rFB and rPCE were prepared as described in Example 1, and crude LAL was prepared as described in Example 2. LAL reagents were generated containing diluted crude LAL, rPCE, and rFB as shown in TABLE 4, and were lyophilized.

[0166] LAL reagent activity was assayed generally as described in Example 2. Results are shown in FIG. 4 and TABLE 5. FIG. 4 shows the onset time at each indicated endotoxin concentration for the reagents, and TABLE 5 shows information on regression curves that result from fitting the data shown in FIG. 4. All formulations showed a dose-dependent response to endotoxin.

TABLE 4

Reagent No.	Final concentration		
	Crude LAL (%)	rFB (U/mL)	rPCE (U/mL)
1	10	0.2	50
2	10	0.2	100
3	10	0.1	50
4	10	0.1	100

TABLE 5

Item	No. 1	No. 2	No. 3	No. 4
Slope	-0.222	-0.223	-0.222	-0.221
Y-intercept	2249	2101	2440	2257
r	-0.999	-1.00	-0.998	-0.999

[0167] These results indicate that hybrid amebocyte lysates (including, e.g., diluted crude LAL, rFB, and/or rPCE) maintain activity following lyophilization.

Example 5

[0168] This example describes the preparation of LAL reagents containing diluted crude LAL, recombinant *Limulus polyphemus* factor B (rFB), and recombinant *Limulus polyphemus* pro-clotting enzyme (rPCE) and their use in kinetic turbidimetric (KTA) or gel-clot assays.

[0169] rFB and rPCE were prepared as described in Example 1, and crude LAL was prepared as described in Example 2. LAL reagents were generated containing 0.5 U/mL rFB, 100 U/mL rPCE, and 19% crude LAL (which is about half amount of crude lysate in a typical KTA LAL reagent). KTA and gel-clot assays were performed according to the Bacterial Endotoxins Test as described in the United States Pharmacopeia (USP).

[0170] Results for the KTA assay are shown in FIG. 5A and for the gel-clot assay are shown in FIG. 5B. The reagent exhibited a dose dependent response to RSE in the KTA assay, and an endpoint of 0.015 EU/mL in the gel-clot assay.

[0171] Together, these results show that the addition of recombinant proteins (e.g., rFB and/or rPCE) to a native amebocyte lysate (e.g., native LAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KTA or gel-clot assay) while maintaining sensitivity of the amebocyte lysate.

Example 6

[0172] This example describes the preparation of LAL reagents containing further diluted crude LAL, recombinant *Limulus polyphemus* factor B (rFB), and recombinant *Limulus polyphemus* pro-clotting enzyme (rPCE) and their use in kinetic turbidimetric (KTA) or gel-clot assays.

[0173] rFB and rPCE were prepared as described in Example 1, and crude LAL was prepared as described in Example 2. LAL reagents were generated containing 0.5 U/mL rFB, 100 U/mL rPCE, and 12% crude LAL (which is about 30% the amount of crude lysate in a typical KTA LAL reagent). LAL reagent activity was assayed generally as described in Example 5.

[0174] Results for the KTA assay are shown in FIG. 6A and for the gel-clot assay are shown in FIG. 6B. The LAL reagent exhibited a dose dependent response to RSE in the KTA assay, but did not achieve an endpoint in this gel-clot assay.

[0175] Together, these results show that the addition of recombinant proteins (e.g., rFB and/or rPCE) to a native amebocyte lysate (e.g., native LAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KTA) while maintaining sensitivity of the amebocyte lysate.

Example 7

[0176] Natural environmental endotoxins (NEE), including endotoxins in water samples, are different from purified endotoxin, such as RSE prepared from *E. coli*, and often show low reactivity to recombinant reagents including recombinant Factor C reagents and recombinant LAL (Dubczak et al. (2021) EUR. J. PHARM. SCI. 159:105716). This example describes the preparation of LAL reagents containing diluted crude LAL, recombinant *Limulus polyphemus* factor B (rFB), and recombinant *Limulus polyphemus* pro-clotting enzyme (rPCE) and their use in the detection of NEEs.

[0177] The following reagents were tested in this Example: (i) a fully recombinant LAL, including 20 U/mL rFC, 0.3 U/mL rFB, and 350 U/mL rPCE ("rLAL"), (ii) a hybrid LAL including diluted (10%) crude LAL, 0.1 U/mL rFB, and 100 U/mL rPCE ("hybrid"), (iii) a first commercially available recombinant factor C (PyroGene, Lonza, "rFC #1"), (iv) a second commercially available recombinant factor C (EndoZyme, BioMerieux, "rFC #2"), (v) a first commercially available native LAL reagent (Endochrome-K, Charles River), and (vi) a second commercially available native LAL reagent (Kinetic QCL, Lonza). For rLAL and hybrid, rFC, rFB and rPCE were prepared as described in Example 1, crude LAL was prepared as described in Example 2, and activity was assayed generally as described in Example 2. All commercially available reagents were used according to manufacturer's instructions.

[0178] The commercially available native LAL reagents (Endochrome-K and Kinetic QCL) were used as a positive control. NEE in 114 water samples was detected using both

Endochrome-K and Kinetic QCL, and the average of these two values was set to 100%. rLAL, hybrid, rFC #1, and rFC #2 were also used to detect NEE in the same 114 water samples, and the results were compared to those obtained using Endochrome-K and Kinetic QCL. According to the criteria in the Bacterial Endotoxins Test (BET) in pharmacopoeias, each reagent was considered to agree with the Endochrome-K and Kinetic QCL average if it differed by no more than a factor of 2 (i.e., ranged from 50% to 200%). The results are shown in TABLE 6.

TABLE 6

Criteria	rFC #1		rFC #2		rLAL		Hybrid	
	Sub-total	Ratio	Sub-total	Ratio	Sub-total	Ratio	Sub-total	Ratio
<50%	93	81.6%	111	97.4%	44	38.6%	1	0.9%
50%-200%	21	18.4%	3	2.6%	70	61.4%	110	96.5%
>200%	0	0.0%	0	0.0%	0	0.0%	3	2.6%
Total	114	100%	114	100%	114	100%	114	100%

[0179] As shown in TABLE 6, the hybrid lysate showed 96.5% agreement with the native LAL reagents (Endochrome-K and Kinetic QCL) while rLAL, rFC #1, and rFC #2 showed high rates of failure. Notably, rFC #1 and rFC #2 reagents failed on the lower side more than 80%, suggesting a high rate of potential false negatives when using these reagents.

[0180] Together, these results indicate that hybrid amebocyte lysate reagents (including, e.g., diluted crude LAL, rFB, and/or rPCE) are able to detect NEE at levels comparable to native LAL reagents, and are superior at detecting NEE relative to fully recombinant reagents.

Example 8

[0181] This example describes the addition of recombinant *Tachypleus tridentatus* factor B, and/or pro-clotting enzyme to crude *Tachypleus* amebocyte lysate (TAL), and the resulting endotoxin detection activity.

[0182] Recombinant *Tachypleus tridentatus* factor B (rFB) and proclotting enzyme (rPCE) were prepared using the same cloning, expression, and purification methods described in Example 1. The DNA sequence encoding *Tachypleus tridentatus* factor B is depicted in SEQ ID NO: 13 (and the corresponding amino acid sequence is depicted in SEQ ID NO: 15). The DNA sequence encoding *Tachypleus tridentatus* pro-clotting enzyme is depicted in SEQ ID NO: 16 (and the corresponding amino acid sequence is depicted in SEQ ID NO: 18).

[0183] Commercially available TAL reagent (Zhanjiang A & C Biological Ltd., China) was reconstituted with LAL reagent water and diluted two-fold in a buffer containing 80 mM HEPES buffer (pH 7.6), 1.6% NaCl, 20 mM MgSO₄, and 1 mM Ac-Ilu-Glu-Gly-Arg-pNA. This reagent is referred to as "100% TAL". A hybrid reagent, referred to as "50% TAL-Hybrid", was prepared by diluting 100% TAL two-fold and adding 0.1 U/mL rFB and 100 U/mL rPCE. A control reagent, referred to as "50% TAL" was also prepared by diluting 100% TAL two-fold without the addition of any recombinant proteins.

[0184] 100% TAL, 50% TAL, and 50% TAL-Hybrid were tested in a KCA assay (generally as described in Example 2).

Results are shown in FIG. 7. As depicted, the sensitivity of 50% TAL was lower than 100% TAL (with 50% TAL having 25% of the sensitivity of 100% TAL). However, 50% TAL-Hybrid showed similar sensitivity as 100% TAL (with 50% TAL-Hybrid having 79% of the sensitivity of 100% TAL).

[0185] 100% TAL, 50% TAL, rLAL (as described in Example 7) and 50% TAL-Hybrid were also used to detect NEE in one of the water samples from Example 7. Results are shown in TABLE 7. Endotoxin values measured using 100% TAL were set to 100%. As depicted, 50% TAL-Hybrid had comparable activity to 100% TAL, and greater activity than 50% TAL or rLAL.

TABLE 7

Reagent	Relative Activity
100% TAL	100%
50% TAL	57%
rLAL	9%
50% TAL Hybrid	88%

[0186] Together, these results show that the addition of rFB and/or rPCE to a native amebocyte lysate (e.g., native TAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KCA assay) while maintaining, or in certain instances even increasing, sensitivity of the amebocyte lysate.

Example 9

[0187] This example describes the addition of recombinant *Limulus polyphemus* factor B, and/or pro-clotting enzyme to crude *Tachypleus* amebocyte lysate (TAL), and the resulting endotoxin detection activity.

[0188] Recombinant *Limulus polyphemus* factor B (rFB) and proclotting enzyme (rPCE) were prepared as described in Example 1. Commercially available TAL reagent (Zhanjiang A & C Biological Ltd., China) was reconstituted with LAL reagent water and diluted two-fold in a buffer containing 80 mM HEPES buffer (pH 7.6), 1.6% NaCl, 20 mM MgSO₄, and 1 mM Ac-Ilu-Glu-Gly-Arg-pNA. This reagent is referred to as "100% TAL". A hybrid reagent, referred to as "50% TAL-rLP Hybrid", was prepared by diluting 100% TAL two-fold and adding 0.1 U/mL *Limulus polyphemus* rFB and 100 U/mL *Limulus polyphemus* rPCE. A control reagent, referred to as "50% TAL" was also prepared by diluting 100% TAL two-fold without the addition of any recombinant proteins.

[0189] 100% TAL, 50% TAL, and 50% TAL-rLP Hybrid were tested in a KCA assay (generally as described in Example 2). Results are shown in FIG. 9. As depicted, the sensitivity of 50% TAL was lower than 100% TAL (with 50% TAL having 38% of the sensitivity of 100% TAL). However, 50% TAL-rLP Hybrid showed greater sensitivity than 100% TAL (with 50% TAL-rLP Hybrid having 113% of the sensitivity of 100% TAL).

[0190] 100% TAL, 50% TAL, rLAL (as described in Example 7) and 50% TAL-rLP were also used to detect NEE in one of the water samples from Example 7. Results are shown in TABLE 8. Endotoxin values measured using 100% TAL were set to 100%. As depicted, 50% TAL-rLP had improved activity relative to 50% TAL or rLAL.

TABLE 8

Reagent	Relative Activity
100% TAL	100%
50% TAL	68%
rLAL	9%
50% TAL-rLP Hybrid	77%

[0191] Together, these results show that the addition of rFB and/or rPCE (e.g., *Limulus* rFB and/or rPCE) to a native amebocyte lysate (e.g., native TAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KCA assay) while maintaining, or in certain instances even increasing, sensitivity of the amebocyte lysate.

Example 10

[0192] This example describes the addition of recombinant *Tachypleus tridentatus* factor B, and/or pro-clotting enzyme to crude *Limulus* amebocyte lysate (LAL), and the resulting endotoxin detection activity.

[0193] Recombinant *Tachypleus tridentatus* factor B (rFB) and proclotting enzyme (rPCE) were prepared as described in Example 8. Crude LAL was prepared as described in Example 2. This reagent is referred to as "100% LAL". A hybrid reagent, referred to as "50% LAL-rT Hybrid", was prepared by diluting 100% LAL two-fold and adding 0.1 U/mL *Tachypleus tridentatus* rFB and 100 U/mL *Tachypleus tridentatus* rPCE. A control reagent, referred to as "50% LAL" was also prepared by diluting 100% LAL two-fold without the addition of any recombinant proteins.

[0194] 100% LAL, 50% LAL, and 50% LAL-rT Hybrid were tested in a KCA assay (generally as described in Example 2). Results are shown in FIG. 10. As depicted, the sensitivity of 50% LAL was lower than 100% LAL (with 50% LAL having 77% of the sensitivity of 100% LAL). However, 50% LAL-rT Hybrid showed greater sensitivity than 100% LAL (with 50% LAL-rT Hybrid having 129% of the sensitivity of 100% LAL).

[0195] 100% LAL, 50% LAL, rLAL (as described in Example 7) and 50% LAL-rT Hybrid were also used to detect NEE in one of the water samples from Example 7. Results are shown in TABLE 9. Endotoxin values measured using 100% LAL were set to 100%. As depicted, 50% LAL-rT Hybrid had comparable activity to 100% LAL, and greater activity than 50% LAL or rLAL.

TABLE 9

Reagent	Relative Activity
100% LAL	100%
50% LAL	66%
rLAL	9%
50% LAL-rTAL Hybrid	91%

[0196] Together, these results show that the addition of rFB and/or rPCE (e.g., *Tachypleus tridentatus* rFB and/or rPCE) to a native amebocyte lysate (e.g., native LAL) can reduce the amount of native amebocyte lysate required to detect endotoxin (e.g., in a KCA assay) while maintaining, or in certain instances even increasing, sensitivity of the amebocyte lysate.

Example 11

[0197] This example provides a comparison of two forms of *Limulus* Factor C expressed in cell lines such that one cell line produced Factor C containing terminal sialic acid glycosylation, and the other produced Factor C without terminal sialic acid glycosylation, and demonstrates their respective biological activities in the presence of different salts.

[0198] The *Limulus* Factor C gene was expressed in two different host cell lines derived from HEK 293 cells. The first cell line was made using the Thermo Fisher Freestyle 293 system and produces expressed proteins fully glycosylated with the usual terminal sialic acid. The second cell line was derived from HEK 293 Gn TI⁻ cells, which lacks the enzymes necessary for addition of the terminal sialic acid.

[0199] *Limulus* Factor C (rFC) derived from HEK293 cells and HEK293 GnTI⁻ cells was prepared as follows. The *Limulus* Factor C gene was cloned into both HEK293 cells and HEK293 GnTI⁻ cells. Cultures of each were grown in Thermo Fisher Freestyle 293 medium according to manufacturer's instructions. Expressed Factor C from both lines were collected from the medium and concentrated by tangential flow filtration (TFF) for use in all experiments.

[0200] For gel electrophoresis, standard SDS polyacrylamide gel electrophoresis was performed, then stained for protein with SYPRO Orange. Fetuin, a glycoprotein, was included as a positive control for sialic acid.

[0201] For Western Blotting, the resulting gel was transferred to a nitrocellulose (NC) membrane by electroblotting and stained for sialic acid content by reaction with the sialic acid binding lectin from *Maackia amurensis*. The following protocol was used as set forth in TABLE 10.

TABLE 10

Materials		
NC membrane from electroblotting		
Biotinylated <i>Maackia amurensis</i> Lectin II	Vector Lab	1 mg/ml
Avidin-Horse Radish Peroxidase (HRP)	BioRad	1:1000
HRP Conjugate Substrate Kit	BioRad	
1% Casein/1x TBS		
Blocker	BioRad	
TBS	BioRad	
TTBS	BioRad	
Procedure		
Blocking:	Rinse NC membrane with TBS	5 min × 2
	Add 1% Casein/1x TBS	shake 1 hr/room temp.
1 st Reaction:	Prepare Biotin-Lectin solution (400 uL TBS)	shake 1 hr/room temp.
	Rinse NC membrane with TTBS	
2 nd Reaction:	Add 20 uL Avidin-HRP in 20 mL TBS (1:1000)	shake 1 hr/room temp.
	Rinse NC membrane with TTBS	10 min. × 3
	Rinse NC membrane with TBS	10 min. × 2
Color Development:	Mix from HRP Conjugate Substrate Kit	
	HRP development buffer 1x 20 mL	
	Reagent A, 0.12 mL	
	Reagent B, 4.0 mL	
	After color develops, rinse w/clean water, photograph	

[0202] Results of the gel electrophoresis are shown in FIG. 11, and the results of the western blot are shown in FIG. 12. The bands of Factor C are circled in each image. A comparison of FIG. 11 with FIG. 12 demonstrates the

terminal sialic acid content of Factor C from HEK293 versus HEK293 GnTI⁻ cell lines. While the SYPRO Orange staining in FIG. 11 shows that Factor C is present in Lanes 2 and 3 along with the control Fetuin protein in Lane 4, FIG. 12 shows that the Factor C produced by the HEK 293 clone (Lane 3) was the only significant sialic acid staining other than the Fetuin positive control (Lane 4). No sialic acid binding was observed in the GnTI⁻ strains where Factor C migrates (Lane 2.)

[0203] In another experiment, a recombinant *Limulus* amoebocyte lysate (LAL) was prepared using recombinant Factor C (rFC) derived from HEK293 cells (HEK293) or HEK293 GnTI⁻ cells (GnTI⁻). Recombinant Factor B (rFB) and recombinant Proclotting Enzyme (rPCE) were added to standard formulation excipients for LAL, containing 0.1 M Tris-HCl buffer, pH 8.0. Then, rFC from HEK293 or GnTI⁻ was added to the solution to achieve functional recombinant LAL, one containing rFC from the HEK293 strain and one containing rFC from the GnTI⁻ strain lacking terminal sialic acid glycosylation.

[0204] Standard curves were established using US Reference Standard Endotoxin (RSE) at 0, 0.025, 0.05, and 0.1 EU/mL. RSE dilutions (0.1 mL) were mixed with the same volume of rLAL on a microplate. The absorbance change rates (mAbs/min) of the reaction mixtures were measured after 30 minutes reaction and the results are shown in FIGS. 13 and 14. Good dose-responses were observed with both HEK293 and GnTI⁻ rFCs (FIG. 13). FIG. 14 shows the absorbance change rates for each endotoxin concentration. Reproducibility was also good (CV<5.3%).

[0205] Additionally, residual activity (endotoxin recovery) of endotoxin in salt solutions was measured. Endotoxin (RSE) was added to salt solutions at 0.05 EU/mL. The salt solutions used were 1.25% NaCl, 2.5% NaCl, 16 mM MgSO₄, 2.5 mM CaCl₂, 21 mM sodium citrate, and 52 mM sodium hydrogen carbonate. The residual activity was calculated by the averaged absorbance change (mAbs/min) with a salt solution containing 0.05 EU/mL by that with 0.05 EU/mL RSE.

[0206] FIG. 15 shows the salt effects on the residual activity (Endotoxin Recovery) of spiked endotoxin as measured in NaCl, MgSO₄, and CaCl₂ solutions. The salt solutions were: 1—1.25% (214 mM) NaCl; 2—2.5% (428 mM)

NaCl; 3—16 mM MgSO₄; and 4—2.5 mM CaCl₂. There was not a significant difference in endotoxin recovery except for 16 mM MgSO₄. The endotoxin recoveries in 16 mM MgSO₄ were 113% and 92%. Considering the variability and the official acceptance range (50%-200%), this difference is negligible in the endotoxin measurement.

[0207] FIG. 16 shows the salts' effects on the residual activity (Endotoxin Recovery) of spiked endotoxin as measured in NaCl, MgSO₄, CaCl₂, sodium citrate and sodium hydrogen carbonate solutions. The salt solutions were: 1—1.25% (214 mM) NaCl; 2—2.5% (428 mM) NaCl; 3—16 mM MgSO₄; 4—2.5 mM CaCl₂; 5—21 mM sodium citrate; and 6—52 mM sodium hydrogen carbonate. The results demonstrate that there was not a significant difference in endotoxin recovery between rLALs using rFCs expressed in HEK293 or GnTI⁻ cell lines. The endotoxin recoveries in 16 mM MgSO₄ were 87% and 75%. Considering the variability and the official acceptance range (50%-200%), this difference is negligible in the endotoxin measurement.

[0208] Equivalent biological activity was observed in Factor C produced by either strain HEK293 or GnTI⁻, regardless of sialic acid content. There was no significant difference in salt effect between rLALs containing rFCs produced in HEK293 and GnTI⁻ cell lines. The difference between these rFCs is the existence of terminal sialic acids. GnTI⁻ cells lack N-acetylglucosaminyltransferase, and are unable to synthesize complex N-glycans.

INCORPORATION BY REFERENCE

[0209] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0210] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

SEQ ID NO: 1

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(Residues 1-25 shown in bold represent the signal sequence)

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(Residues 1-25 shown in bold represent the signal sequence)

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(Residues 1-28 shown in bold represent the signal sequence)

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SEQ ID NO: 10

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SEQ ID NO: 11

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VRNPPDNQPTIDYSCSPGFKLKGVARISCLPNGQWSFPFKCIRECAKVSSPEHGKVNAPSGNIM
IEGATLRFSCDSPYILIGQETLTQGNQWSGQIPQCKKLVPDLPVNHAEHQVIGVEQKY
GQFPQGTEVYTCSGNYFLMGFNTLKNPDGWSGSGQPSVKVADREVDSCSAVDLDDVGE
VRIHCPAGCSLTAGTVWGTAIYHELSSVCRAAIIHAGKLPNSGGAVHVNNNGPYSDFLGSDLNGI
KSEELKSLARSFRPDYVSSSTAGRSGCPDGWFEVENCYVYTSKQRAWERAQGVCTNMAARLAV
LDKDLIPSSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLNDLNTFWASGEPGNETN

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CVYLDIRDLQPVWKTSCFPSSFACMMDLSDRNKAKCDDPGPLENGHATLHGQSIDGFYAGS
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 GSPFLRLPRLPLPLARAAPPPKPRSSQPSVTLASKVKLPEGHYRVGSRAIYTCESRYEELG
 SQRRCDNSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIGQWPQAGISRWLADHNMWFLQ
 CGGSLNNEKWIIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDSRDDYVQVREALEIHVNPNY
 DPGNLNFDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEGTLAVVTGWGLNENNTYSEMIQQ
 AVLPPVVAASTCEEYKEADLPLTVTENMFACAGYKKGRYDACSGDSGGPLVFADDSRTERRWVLE
 GIVSWGSPSGCGKANQYGGFTKVNVLFSWIRQFI

(Residues 1-21 in bold represent the signal sequence)

SEQ ID NO: 12

MKFLVNLVALFMVYIYISYIYARGVDLGLCDETRFECKCGDPGYVFNVPKQCTYFYRWRPYCKP
 CDDLEAKDICKPKYKRCQECKAGLDSVTCPPNKYGTWCSGECQCKNGGICDQRTGACTCRDRYE
 GAHCEILKGCPLLPDSQVQEVNPPDPNPQTIDYSCSPGFKLGVARISCLPNGQWSSFPKCI
 RECAKVSSPEHGKVNAPSGNMIEGATLRFSCDSPYYLIGQETLTCQNGQWWSGQIPQCKKLVPFC
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 ADREVDCDKAVDFLDDVGEVRIHCPAGCSLTAGTVWGTAIYHELSSVCRAAIHAGKLPNSGG
 AVHVNNNGPYSDFLGSDLNGIKSEELKSLARSFRFDYVSSSTAGRSGCPDGWFEVEENCYVTS
 KQRAWERAQGVCTNMAARLAVLDKDLIPSSLTETLRGKGLTTTWIGLHRLDAEKPFWELMDRS
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 PLENGHATLHGQSIDGFYAGSSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKVITCQNP
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 HYRVGSRAIYTCESRYEELGSGRRCDNSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIG
 QWPWQAGISRWLADHNMWFLQCGGSLNNEKWIIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDS
 SRDDYVQVREALEIHVNPNYDPGNLFDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEGT
 LAVVTGWGLNENNTYSEMIQQAVLPPVVAASTCEEYKEADLPLTVTENMFACAGYKKGRYDACSG
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SEQ ID NO: 13

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 CCGATTCTGCCCGCATCTCCACCCGCAAACTCCACTGGTGGCAGGGGCATCATTCGCGGTG
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 CGGGCGGTTCCACTGCGCTGGTTCGATCATCTCCAACAAGTACATCTGTCCGCGCACATGCC
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 TCAAGCGCGGACAGGAATACCCCGTGAAGGACGTGATAATCCACCCCACTACGTGGAAAAGGA
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 CCCATCTGCCCTCCCTGACCCCGAGACAGTGACCGACCCACTCAAAGACCGCATCGTGACTGCAG
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SEQ ID NO: 14

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VEAKIGAWPWMAAVFVNFGIGRFHCAGSIISNKYILSAHAFLIGGRKLTPTRLAVRVGGHYI
KRQGEYPVKDVI IHPHYVEKENYNDIAIIELKEELNFTDLVNPICLPDPETVTDPLKDRIVTAA
GWGDLDFSGPRSQVLREVSIPVVPDKCDQAYEKLNTPSLKNGITNNFLCAGLEEGGKDACQGD
SGGPLMLVNNTRWIVGVVSFGHKCAEEGYPGVYSRVASYLDWI AKVTNSLDHAVTN

(Residues 1-22 represent the signal sequence)

SEQ ID NO: 15

MDMRVPAQLLGLLLWFPGRSRCVGVLPKTRNDNECTARGGLKGSCKSLIDCPSVLATLKDSFP
VVC SWNGRFQPIVCCPDAIAPPVTTTAVTVISTKEPKLRLHISGCGKRKVKIDITTVGRSGS
PILPPISTPQNSTGGRGIIAGGVEAKIGAWPWMAAVFVNFGIGRFHCAGSIISNKYILSAHA
FLIGGRKLTPTRLAVRVGGHYIKRQGEYPVKDVI IHPHYVEKENYNDIAIIELKEELNFTDLVN
PICLPDPETVTDPLKDRIVTAAAGWDLDFSGPRSQVLREVSIPVVPDKCDQAYEKLNTPSLKN
GITNNFLCAGLEEGGKDACQGD SGGPLMLVNNTRWIVGVVSFGHKCAEEGYPGVYSRVASYLD
WIAKVTNSLDHAVTN

SEQ ID NO: 16

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SEQ ID NO: 17
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 KSSHVISSTQAPPETTTTTERPPKQIPPNLPEVCGIHNTTTTRIIGGREAPIGAWPMTAVYIKQ
 GGIRSVQCGGALVTNRHVI TASHCVVNSAGTDVMPADVFSVRLGEHNLSTDDDSNPIDFAVTS
 VKHHEHFVLATYLNDAIILTLNDTVTFTDRIRPICLPYRKLRYDDLAMRKPFITGWGTTAFNGP
 SSAVLREVOLPIWEHEACRQAYEKDLNITNVYMCAGFADGGKDACQDGGGPMMLPVKTGEFYLL
 IGIVSFGKKCALPGFPGVYTKVTEFLDWIAEHMV

(Residues 1-21 represent the signal sequence)

SEQ ID NO: 18
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 YNLLKESICGFEGITPKVCCPKSSHVISSTQAPPETTTTTERPPKQIPPNLPEVCGIHNTTTTRI
 IGGREAPIGAWPMTAVYIKQGGIRSVQCGGALVTNRHVI TASHCVVNSAGTDVMPADVFSVRL
 GEHNLSTDDDSNPIDFAVTSVKHHEHFVLATYLNDAIILTLNDTVTFTDRIRPICLPYRKLRY
 DDLAMRKPFITGWGTTAFNGPSSAVLREVQLPIWEHEACRQAYEKDLNITNVYMCAGFADGGKD
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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 19

<210> SEQ ID NO 1

<211> LENGTH: 3063

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 1

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cggcgtggg tgctcgaggg tattgtgtcc tggggatccc ccaacggatg cgggaagtcc	3000
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tga	3063

<210> SEQ ID NO 2
 <211> LENGTH: 995
 <212> TYPE: PRT
 <213> ORGANISM: Limulus polyphemus
 <400> SEQUENCE: 2

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Tyr	Phe	Tyr	Arg	Trp	Arg	Pro	Tyr	Cys	Lys	Pro	Cys	Asp	Lys	Leu	Glu	35	40	45	
Ala	Lys	Asp	Val	Cys	Pro	Lys	Tyr	Lys	Arg	Cys	Gln	Glu	Cys	Arg	Ala	50	55	60	
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Cys	Ser	Gly	Glu	Cys	Gln	Cys	Lys	Asn	Gly	Gly	Ile	Cys	Asp	Gln	Arg	85	90	95	
Thr	Gly	Ala	Cys	Thr	Cys	Arg	Asp	Arg	Tyr	Glu	Gly	Val	His	Cys	Glu	100	105	110	
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Val	Lys	Asn	Pro	Pro	Asn	Asp	Pro	Gln	Thr	Ile	Asp	Tyr	Ser	Cys	Ser	130	135	140	
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Gly	Gln	Trp	Ser	Ser	Phe	Pro	Pro	Lys	Cys	Ile	Arg	Glu	Cys	Ser	Met	165	170	175	
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Ile	Gly	Gln	Glu	Thr	Leu	Thr	Cys	Gln	Gly	Asn	Gly	Gln	Trp	Ser	Gly	210	215	220	
Gln	Ile	Pro	Gln	Cys	Gln	Lys	Leu	Val	Phe	Cys	Pro	Asp	Leu	Asp	Pro	225	230	235	240
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Gly	Gln	Phe	Pro	Gln	Gly	Thr	Glu	Val	Thr	Tyr	Thr	Cys	Thr	Gly	Asn	260	265	270	
Tyr	Phe	Leu	Met	Gly	Leu	Asp	Thr	Leu	Lys	Cys	Asn	Pro	Asp	Gly	Ser	275	280	285	
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Asn	Cys	Asp	Ser	Lys	Ala	Val	Asp	Phe	Leu	Asp	Asp	Val	Gly	Glu	Pro	305	310	315	320
Val	Arg	Ile	His	Cys	Pro	Ala	Gly	Cys	Ser	Leu	Thr	Ala	Gly	Thr	Val	325	330	335	
Trp	Gly	Thr	Ala	Ile	Tyr	His	Glu	Leu	Ser	Ser	Val	Cys	Arg	Ala	Ala	340	345	350	
Ile	His	Ala	Gly	Lys	Val	Pro	Asn	Ser	Gly	Gly	Ala	Val	His	Val	Val	355	360	365	
Asn	Asn	Gly	Pro	Tyr	Ser	Asp	Phe	Leu	Ala	Ser	Asp	Leu	Asn	Gly	Ile	370	375	380	
Lys	Ser	Asp	Glu	Leu	Lys	Ser	Leu	Ala	Gln	Ser	Phe	Arg	Phe	Asp	Tyr	385	390	395	400

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		500						505					510		
Asn	Cys	Val	Tyr	Leu	Asp	Ile	Gln	Asp	Gln	Leu	Gln	Pro	Val	Trp	Lys
		515					520					525			
Thr	Lys	Ser	Cys	Phe	Gln	Pro	Ser	Ser	Phe	Val	Cys	Met	Met	Asp	Leu
	530					535					540				
Ser	Asp	Lys	Asn	Lys	Ala	Lys	Cys	Lys	Asp	Pro	Gly	Pro	Leu	Glu	Asn
545				550					555					560	
Gly	His	Ala	Lys	Leu	His	Gly	Gln	Ser	Ile	Asp	Gly	Phe	Tyr	Ala	Gly
			565					570						575	
Ser	Ser	Val	Arg	Tyr	Ser	Cys	Glu	Val	Leu	His	Tyr	Leu	Ser	Gly	Thr
		580						585					590		
Glu	Thr	Val	Ser	Cys	Thr	Ser	Asn	Gly	Thr	Trp	Ser	Ala	Pro	Lys	Pro
	595						600					605			
Arg	Cys	Ile	Lys	Val	Ile	Thr	Cys	Gln	Thr	Pro	Pro	Val	Pro	Ser	Tyr
	610					615				620					
Gly	Ser	Val	Asp	Ile	Lys	Pro	Pro	Ser	Arg	Thr	Asn	Ser	Ile	Ser	Arg
625				630					635					640	
Val	Gly	Ser	Pro	Phe	Leu	Arg	Leu	Pro	Arg	Leu	Pro	Leu	Pro	Leu	Ala
			645					650					655		
Arg	Ala	Ala	Gly	Pro	Pro	Pro	Lys	Pro	Arg	Ser	Ala	Pro	Pro	Ser	Thr
		660					665					670			
Val	Asp	Leu	Ser	Ser	Lys	Val	Lys	Leu	Pro	Glu	Gly	His	Tyr	Arg	Val
	675					680						685			
Gly	Ser	Gln	Ala	Ile	Tyr	Thr	Cys	Glu	Ser	Arg	Tyr	Tyr	Glu	Leu	Leu
	690					695				700					
Gly	Ser	Gln	Gly	Arg	Arg	Cys	Asp	Ser	Asn	Gly	Lys	Trp	Ser	Gly	Arg
705				710					715					720	
Pro	Ala	Ser	Cys	Ile	Pro	Val	Cys	Gly	Arg	Ser	Asp	Ser	Pro	Arg	Ser
			725					730					735		
Pro	Phe	Ile	Val	Asn	Gly	Asn	Ser	Thr	Glu	Ile	Gly	Gln	Trp	Pro	Trp
		740						745					750		
Gln	Ala	Gly	Ile	Ser	Arg	Trp	Leu	Ala	Asp	His	Asn	Met	Trp	Phe	Leu
	755					760						765			
Gln	Cys	Gly	Gly	Ala	Leu	Leu	Asn	Glu	Lys	Trp	Ile	Ile	Thr	Ala	Ala
	770					775					780				
His	Cys	Val	Thr	Tyr	Ser	Ala	Thr	Ala	Glu	Ile	Ile	Asp	Pro	Ser	Gln
785					790					795				800	
Phe	Lys	Phe	Tyr	Leu	Gly	Lys	Tyr	Tyr	Arg	Asp	Asp	Ser	Lys	Asp	Asp

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805					810					815						
Asp	Tyr	Val	Gln	Val	Arg	Glu	Ala	Ile	Glu	Ile	His	Val	Asn	Pro	Asn	
820					825					830						
Tyr	Asp	Pro	Gly	Asn	Leu	Asn	Phe	Asp	Ile	Ala	Leu	Ile	Gln	Leu	Lys	
835					840					845						
Thr	Ser	Val	Ala	Leu	Thr	Thr	Arg	Val	Gln	Pro	Ile	Cys	Leu	Pro	Thr	
850					855					860						
Asp	Leu	Thr	Thr	Arg	Glu	Asn	Leu	Lys	Glu	Gly	Ala	Leu	Ala	Val	Val	
865					870					875					880	
Thr	Gly	Trp	Gly	Leu	Asn	Glu	Asn	Asn	Thr	Tyr	Ser	Glu	Met	Ile	Gln	
885					890					895						
Gln	Ala	Val	Leu	Pro	Val	Val	Ala	Ala	Ser	Thr	Cys	Glu	Gln	Gly	Tyr	
900					905					910						
Gln	Asp	Ser	Gly	Leu	Pro	Leu	Thr	Val	Thr	Glu	Asn	Met	Phe	Cys	Ala	
915					920					925						
Gly	Tyr	Lys	Gln	Gly	Arg	Tyr	Asp	Ala	Cys	Ser	Gly	Asp	Ser	Gly	Gly	
930					935					940						
Pro	Leu	Val	Phe	Ala	Asp	Asp	Ser	Arg	Thr	Asp	Arg	Arg	Trp	Val	Leu	
945					950					955					960	
Glu	Gly	Ile	Val	Ser	Trp	Gly	Ser	Pro	Asn	Gly	Cys	Gly	Lys	Ser	Asn	
965					970					975						
Gln	Tyr	Gly	Gly	Phe	Thr	Lys	Val	Asn	Val	Phe	Leu	Ser	Trp	Ile	Arg	
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Gln	Phe	Ile														
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<210> SEQ ID NO 3																
<211> LENGTH: 1020																
<212> TYPE: PRT																
<213> ORGANISM: Limulus polyphemus																
<400> SEQUENCE: 3																
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1		5				10				15						
Ala	Gln	Lys	Met	Arg	Pro	Val	Gln	Ser	Arg	Gly	Val	Asp	Leu	Gly	Leu	
20				25				30								
Cys	Asp	Asp	Thr	Arg	Phe	Glu	Cys	Lys	Cys	Gly	Asp	Pro	Gly	Tyr	Val	
35			40			45										
Phe	Asn	Val	Pro	Ala	Lys	Gln	Cys	Thr	Tyr	Phe	Tyr	Arg	Trp	Arg	Pro	
50		55				60										
Tyr	Cys	Lys	Pro	Cys	Asp	Lys	Leu	Glu	Ala	Lys	Asp	Val	Cys	Pro	Lys	
65		70				75				80						
Tyr	Lys	Arg	Cys	Gln	Glu	Cys	Arg	Ala	Gly	Leu	Asp	Ser	Cys	Val	Ser	
85				90				95								
Cys	Pro	Pro	Asn	Lys	Tyr	Gly	Thr	Trp	Cys	Ser	Gly	Glu	Cys	Gln	Cys	
100				105				110								
Lys	Asn	Gly	Gly	Ile	Cys	Asp	Gln	Arg	Thr	Gly	Ala	Cys	Thr	Cys	Arg	
115				120				125								
Asp	Arg	Tyr	Glu	Gly	Val	His	Cys	Glu	Ile	Leu	Gln	Gly	Cys	Pro	Leu	
130		135				140										
Leu	Gln	Ser	Asp	Pro	Gln	Val	Gln	Glu	Val	Lys	Asn	Pro	Pro	Asn	Asp	
145		150				155				160						

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Pro	Gln	Thr	Ile	Asp	Tyr	Ser	Cys	Ser	Pro	Gly	Phe	Lys	Leu	Lys	Gly
			165						170					175	
Val	Ala	Arg	Ile	Thr	Cys	Leu	Pro	Asn	Gly	Gln	Trp	Ser	Ser	Phe	Pro
			180					185					190		
Pro	Lys	Cys	Ile	Arg	Glu	Cys	Ser	Met	Val	Ser	Ser	Leu	Glu	His	Gly
		195					200					205			
Lys	Val	Asn	Ser	Pro	Ser	Ala	Asp	Leu	Ile	Glu	Gly	Ala	Thr	Leu	Arg
		210				215					220				
Phe	Ser	Cys	Asp	Ser	Pro	Tyr	Tyr	Leu	Ile	Gly	Gln	Glu	Thr	Leu	Thr
					230					235					240
Cys	Gln	Gly	Asn	Gly	Gln	Trp	Ser	Gly	Gln	Ile	Pro	Gln	Cys	Gln	Lys
			245						250					255	
Leu	Val	Phe	Cys	Pro	Asp	Leu	Asp	Pro	Val	Ser	His	Ala	Glu	His	Gln
			260					265					270		
Val	Lys	Ile	Gly	Leu	Glu	Gln	Lys	Tyr	Gly	Gln	Phe	Pro	Gln	Gly	Thr
		275					280					285			
Glu	Val	Thr	Tyr	Thr	Cys	Thr	Gly	Asn	Tyr	Phe	Leu	Met	Gly	Leu	Asp
		290				295					300				
Thr	Leu	Lys	Cys	Asn	Pro	Asp	Gly	Ser	Trp	Ser	Gly	Thr	Gln	Pro	Ser
				310						315					320
Cys	Val	Lys	Val	Ala	Asp	Arg	Glu	Val	Asn	Cys	Asp	Ser	Lys	Ala	Val
			325						330					335	
Asp	Phe	Leu	Asp	Asp	Val	Gly	Glu	Pro	Val	Arg	Ile	His	Cys	Pro	Ala
			340					345					350		
Gly	Cys	Ser	Leu	Thr	Ala	Gly	Thr	Val	Trp	Gly	Thr	Ala	Ile	Tyr	His
		355					360					365			
Glu	Leu	Ser	Ser	Val	Cys	Arg	Ala	Ala	Ile	His	Ala	Gly	Lys	Val	Pro
				370		375					380				
Asn	Ser	Gly	Gly	Ala	Val	His	Val	Val	Asn	Asn	Gly	Pro	Tyr	Ser	Asp
				385		390				395					400
Phe	Leu	Ala	Ser	Asp	Leu	Asn	Gly	Ile	Lys	Ser	Asp	Glu	Leu	Lys	Ser
			405					410						415	
Leu	Ala	Gln	Ser	Phe	Arg	Phe	Asp	Tyr	Val	Ser	Ser	Ser	Thr	Ala	Gly
			420					425					430		
Arg	Lys	Ser	Gly	Cys	Pro	Asp	Gly	Trp	Phe	Glu	Ile	Glu	Glu	Asn	Cys
			435				440					445			
Val	Tyr	Val	Thr	Ser	Lys	Gln	Arg	Ala	Trp	Glu	Arg	Ala	Gln	Gly	Val
			450			455					460				
Cys	Thr	Asn	Met	Ala	Ala	Arg	Leu	Ala	Val	Leu	Asp	Lys	Asp	Val	Ile
				465		470				475				480	
Pro	Ser	Ser	Leu	Thr	Glu	Thr	Leu	Arg	Gly	Lys	Gly	Leu	Ala	Thr	Thr
			485					490						495	
Trp	Ile	Gly	Leu	His	Arg	Leu	Asp	Ala	Asp	Asn	His	Phe	Ile	Trp	Glu
			500				505						510		
Leu	Met	Asp	Arg	Ser	Ser	Val	Ala	Leu	Asn	Asp	Ser	Leu	Thr	Phe	Trp
			515				520					525			
Ala	Pro	Gly	Glu	Pro	Gly	Asn	Glu	Thr	Asn	Cys	Val	Tyr	Leu	Asp	Ile
				530		535					540				
Gln	Asp	Gln	Leu	Gln	Pro	Val	Trp	Lys	Thr	Lys	Ser	Cys	Phe	Gln	Pro
				545		550				555					560
Ser	Ser	Phe	Val	Cys	Met	Met	Asp	Leu	Ser	Asp	Lys	Asn	Lys	Ala	Lys

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565					570					575					
Cys	Lys	Asp	Pro	Gly	Pro	Leu	Glu	Asn	Gly	His	Ala	Lys	Leu	His	Gly
			580						585				590		
Gln	Ser	Ile	Asp	Gly	Phe	Tyr	Ala	Gly	Ser	Ser	Val	Arg	Tyr	Ser	Cys
		595					600					605			
Glu	Val	Leu	His	Tyr	Leu	Ser	Gly	Thr	Glu	Thr	Val	Ser	Cys	Thr	Ser
	610					615					620				
Asn	Gly	Thr	Trp	Ser	Ala	Pro	Lys	Pro	Arg	Cys	Ile	Lys	Val	Ile	Thr
625					630					635				640	
Cys	Gln	Thr	Pro	Pro	Val	Pro	Ser	Tyr	Gly	Ser	Val	Asp	Ile	Lys	Pro
				645					650				655		
Pro	Ser	Arg	Thr	Asn	Ser	Ile	Ser	Arg	Val	Gly	Ser	Pro	Phe	Leu	Arg
			660					665					670		
Leu	Pro	Arg	Leu	Pro	Leu	Pro	Leu	Ala	Arg	Ala	Ala	Gly	Pro	Pro	Pro
	675						680					685			
Lys	Pro	Arg	Ser	Ala	Pro	Pro	Ser	Thr	Val	Asp	Leu	Ser	Ser	Lys	Val
	690					695					700				
Lys	Leu	Pro	Glu	Gly	His	Tyr	Arg	Val	Gly	Ser	Gln	Ala	Ile	Tyr	Thr
705					710					715				720	
Cys	Glu	Ser	Arg	Tyr	Tyr	Glu	Leu	Leu	Gly	Ser	Gln	Gly	Arg	Arg	Cys
				725					730				735		
Asp	Ser	Asn	Gly	Lys	Trp	Ser	Gly	Arg	Pro	Ala	Ser	Cys	Ile	Pro	Val
			740					745					750		
Cys	Gly	Arg	Ser	Asp	Ser	Pro	Arg	Ser	Pro	Phe	Ile	Val	Asn	Gly	Asn
		755					760					765			
Ser	Thr	Glu	Ile	Gly	Gln	Trp	Pro	Trp	Gln	Ala	Gly	Ile	Ser	Arg	Trp
	770					775					780				
Leu	Ala	Asp	His	Asn	Met	Trp	Phe	Leu	Gln	Cys	Gly	Gly	Ala	Leu	Leu
785					790					795				800	
Asn	Glu	Lys	Trp	Ile	Ile	Thr	Ala	Ala	His	Cys	Val	Thr	Tyr	Ser	Ala
				805					810					815	
Thr	Ala	Glu	Ile	Ile	Asp	Pro	Ser	Gln	Phe	Lys	Phe	Tyr	Leu	Gly	Lys
		820						825					830		
Tyr	Tyr	Arg	Asp	Asp	Ser	Lys	Asp	Asp	Asp	Tyr	Val	Gln	Val	Arg	Glu
		835					840					845			
Ala	Ile	Glu	Ile	His	Val	Asn	Pro	Asn	Tyr	Asp	Pro	Gly	Asn	Leu	Asn
	850					855					860				
Phe	Asp	Ile	Ala	Leu	Ile	Gln	Leu	Lys	Thr	Ser	Val	Ala	Leu	Thr	Thr
865					870					875				880	
Arg	Val	Gln	Pro	Ile	Cys	Leu	Pro	Thr	Asp	Leu	Thr	Thr	Arg	Glu	Asn
				885					890					895	
Leu	Lys	Glu	Gly	Ala	Leu	Ala	Val	Val	Thr	Gly	Trp	Gly	Leu	Asn	Glu
		900							905				910		
Asn	Asn	Thr	Tyr	Ser	Glu	Met	Ile	Gln	Gln	Ala	Val	Leu	Pro	Val	Val
		915					920					925			
Ala	Ala	Ser	Thr	Cys	Glu	Gln	Gly	Tyr	Gln	Asp	Ser	Gly	Leu	Pro	Leu
	930					935					940				
Thr	Val	Thr	Glu	Asn	Met	Phe	Cys	Ala	Gly	Tyr	Lys	Gln	Gly	Arg	Tyr
945					950					955				960	
Asp	Ala	Cys	Ser	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Phe	Ala	Asp	Asp
				965					970					975	

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Ser Arg Thr Asp Arg Arg Trp Val Leu Glu Gly Ile Val Ser Trp Gly
 980 985 990

Ser Pro Asn Gly Cys Gly Lys Ser Asn Gln Tyr Gly Gly Phe Thr Lys
 995 1000 1005

Val Asn Val Phe Leu Ser Trp Ile Arg Gln Phe Ile
 1010 1015 1020

<210> SEQ ID NO 4
 <211> LENGTH: 1203
 <212> TYPE: DNA
 <213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 4

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aagggtggac tcaagggcag ctgcaaatcc ctactgact gcccgcgct gctggcgacc    180
cttaaggaca gcttccctgt cgtgtgtagc tggaatggcc ggttccaacc gattgtgtgc    240
tgtccggacg ccgcccgcct ttccgtgaca actaccgtga cactatcgt gccgaccaag    300
gaaactaaga tccctagact gcatacccg ggctgcggga aacggaaagt caacgtggac    360
atcaccacca tcggacggtc cgttagcccg atcctgccgc ccattagcac gtcacaggac    420
ctgaaggggg gccgcggaat cattgccgcg ggagtggaa cgaagattgg ggcgtggccc    480
tggtggcccg cagtgttcgt gaagaacttt ggcattggaa gattccactg cgcgggatcc    540
atcatttcgt ccaagtacat tctgtccgcc gccacgcct tcctgatcgg cggccgcaag    600
ctgaccccca ccgcttggtg cgtgcgcgtg ggccgacact acgtcaagat gggccaagag    660
taccacgtgg aagatgtcat catccacct gactacgtgg aacgggagaa ctacaacgac    720
atcgctatca tcgtgtctga ggaggagctg aacttcaccg acctcgtcgc cccaatctgc    780
ctgccgggac cagagggcgt gaccgattca ctcaagggtc ggagagtgc cgtcgccggt    840
tgggggggacc tcgacttcgc gggcccccg tccaagtgc tgcgggaggt gtccatacca    900
gtggtgccta ttggcgattg caacaaggca ttcagaagc tcaacacctt ggctctgaag    960
aacggaatta ccaagaagtt catctgcgcc gggctggaag aagggggaaa ggacgcgtgc   1020
caggggcatt cgggtggacc cctgatgctg gtcaacaact catcgtggat cgtgaccgga   1080
gtggtgtcct tcggacataa gtgcgccgaa gaagggttcc cgggagtcta caccagggtg   1140
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<210> SEQ ID NO 5
 <211> LENGTH: 375
 <212> TYPE: PRT
 <213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 5

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Gly Gly Leu Lys Gly Ser Cys Lys Ser Leu Thr Asp Cys Pro Ala Val
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Leu Ala Thr Leu Lys Asp Ser Phe Pro Val Val Cys Ser Trp Asn Gly
 35 40 45

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Arg Phe Gln Pro Ile Val Cys Cys Pro Asp Ala Ala Ala Pro Ser Val
 50 55 60
 Thr Thr Thr Val Thr Thr Ile Val Pro Thr Lys Glu Thr Lys Ile Pro
 65 70 75 80
 Arg Leu His Ile Pro Gly Cys Gly Lys Arg Lys Val Asn Val Asp Ile
 85 90 95
 Thr Thr Ile Gly Arg Ser Gly Ser Pro Ile Leu Pro Pro Ile Ser Thr
 100 105 110
 Ser Gln Asp Leu Lys Gly Gly Arg Gly Ile Ile Ala Gly Gly Val Glu
 115 120 125
 Ala Lys Ile Gly Ala Trp Pro Trp Met Ala Ala Val Phe Val Lys Asn
 130 135 140
 Phe Gly Ile Gly Arg Phe His Cys Ala Gly Ser Ile Ile Ser Ser Lys
 145 150 155 160
 Tyr Ile Leu Ser Ala Ala His Ala Phe Leu Ile Gly Gly Arg Lys Leu
 165 170 175
 Thr Pro Thr Arg Leu Ala Val Arg Val Gly Gly His Tyr Val Lys Met
 180 185 190
 Gly Gln Glu Tyr His Val Glu Asp Val Ile Ile His Pro Asp Tyr Val
 195 200 205
 Glu Arg Glu Asn Tyr Asn Asp Ile Ala Ile Ile Val Leu Lys Glu Glu
 210 215 220
 Leu Asn Phe Thr Asp Leu Val Arg Pro Ile Cys Leu Pro Asp Pro Glu
 225 230 235 240
 Ala Val Thr Asp Ser Leu Lys Gly Arg Arg Val Thr Val Ala Gly Trp
 245 250 255
 Gly Asp Leu Asp Phe Ala Gly Pro Arg Ser Gln Val Leu Arg Glu Val
 260 265 270
 Ser Ile Pro Val Val Pro Ile Gly Asp Cys Asn Lys Ala Tyr Gln Lys
 275 280 285
 Leu Asn Thr Leu Ala Leu Lys Asn Gly Ile Thr Lys Lys Phe Ile Cys
 290 295 300
 Ala Gly Leu Glu Glu Gly Gly Lys Asp Ala Cys Gln Gly Asp Ser Gly
 305 310 315 320
 Gly Pro Leu Met Leu Val Asn Asn Ser Ser Trp Ile Val Thr Gly Val
 325 330 335
 Val Ser Phe Gly His Lys Cys Ala Glu Glu Gly Phe Pro Gly Val Tyr
 340 345 350
 Thr Arg Val Val Ser Tyr Leu Glu Trp Ile Ala Lys Val Thr Asn Ser
 355 360 365
 Leu Asp Gln Thr Val Thr Asn
 370 375

<210> SEQ ID NO 6
 <211> LENGTH: 400
 <212> TYPE: PRT
 <213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 6

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 Leu Ser Asn Lys Val Ser Arg Val Gly Ile Ile Phe Pro Lys Thr Gln

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Asn	Asp	Asn	Lys	Gln	Cys	Thr	Ala	Lys	Gly	Gly	Leu	Lys	Gly	Ser	Cys				
35					40					45									
Lys	Ser	Leu	Thr	Asp	Cys	Pro	Ala	Val	Leu	Ala	Thr	Leu	Lys	Asp	Ser				
50					55					60									
Phe	Pro	Val	Val	Cys	Ser	Trp	Asn	Gly	Arg	Phe	Gln	Pro	Ile	Val	Cys				
65					70					75					80				
Cys	Pro	Asp	Ala	Ala	Ala	Pro	Ser	Val	Thr	Thr	Thr	Val	Thr	Thr	Ile				
85					90					95									
Val	Pro	Thr	Lys	Glu	Thr	Lys	Ile	Pro	Arg	Leu	His	Ile	Pro	Gly	Cys				
100					105					110									
Gly	Lys	Arg	Lys	Val	Asn	Val	Asp	Ile	Thr	Thr	Ile	Gly	Arg	Ser	Gly				
115					120					125									
Ser	Pro	Ile	Leu	Pro	Pro	Ile	Ser	Thr	Ser	Gln	Asp	Leu	Lys	Gly	Gly				
130					135					140									
Arg	Gly	Ile	Ile	Ala	Gly	Gly	Val	Glu	Ala	Lys	Ile	Gly	Ala	Trp	Pro				
145					150					155					160				
Trp	Met	Ala	Ala	Val	Phe	Val	Lys	Asn	Phe	Gly	Ile	Gly	Arg	Phe	His				
165					170					175									
Cys	Ala	Gly	Ser	Ile	Ile	Ser	Ser	Lys	Tyr	Ile	Leu	Ser	Ala	Ala	His				
180					185					190									
Ala	Phe	Leu	Ile	Gly	Gly	Arg	Lys	Leu	Thr	Pro	Thr	Arg	Leu	Ala	Val				
195					200					205									
Arg	Val	Gly	Gly	His	Tyr	Val	Lys	Met	Gly	Gln	Glu	Tyr	His	Val	Glu				
210					215					220									
Asp	Val	Ile	Ile	His	Pro	Asp	Tyr	Val	Glu	Arg	Glu	Asn	Tyr	Asn	Asp				
225					230					235					240				
Ile	Ala	Ile	Ile	Val	Leu	Lys	Glu	Glu	Leu	Asn	Phe	Thr	Asp	Leu	Val				
245					250					255									
Arg	Pro	Ile	Cys	Leu	Pro	Asp	Pro	Glu	Ala	Val	Thr	Asp	Ser	Leu	Lys				
260					265					270									
Gly	Arg	Arg	Val	Thr	Val	Ala	Gly	Trp	Gly	Asp	Leu	Asp	Phe	Ala	Gly				
275					280					285									
Pro	Arg	Ser	Gln	Val	Leu	Arg	Glu	Val	Ser	Ile	Pro	Val	Val	Pro	Ile				
290					295					300									
Gly	Asp	Cys	Asn	Lys	Ala	Tyr	Gln	Lys	Leu	Asn	Thr	Leu	Ala	Leu	Lys				
305					310					315					320				
Asn	Gly	Ile	Thr	Lys	Lys	Phe	Ile	Cys	Ala	Gly	Leu	Glu	Glu	Gly	Gly				
325					330					335									
Lys	Asp	Ala	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Met	Leu	Val	Asn				
340					345					350									
Asn	Ser	Ser	Trp	Ile	Val	Thr	Gly	Val	Val	Ser	Phe	Gly	His	Lys	Cys				
355					360					365									
Ala	Glu	Glu	Gly	Phe	Pro	Gly	Val	Tyr	Thr	Arg	Val	Val	Ser	Tyr	Leu				
370					375					380									
Glu	Trp	Ile	Ala	Lys	Val	Thr	Asn	Ser	Leu	Asp	Gln	Thr	Val	Thr	Asn				
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<210> SEQ ID NO 7

<211> LENGTH: 1149

<212> TYPE: DNA

<213> ORGANISM: Limulus polyphemus

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<400> SEQUENCE: 7

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ccggatgacg aagagtcttg tagcaaccgg ttaccaacg acgggatctg caaggacgtg    180
ctgaactgcc gcgatctgct gcagaagaat gactacaacc tcttgaagga atccatctgc    240
ggcttcgaag ggatcacccc taaagtctgc tgtccaaagc aatccattgt gaaccccatc    300
accgaagccc cgctaagac caccactact gagaggcctc cgatccggat cccctccaac    360
ctcccgaagc agtgcggaaa tcggaacatc actaccacta ggatcatcgg cggacaggaa    420
gccactccgg gagcctggcc gtggatggca gccgtgtaca ttaagcaggg ggggattcgg    480
tccgtgcagt gcggtggcgc ccttgtgacc aacagacacg tgatcacgcg ctcacactgc    540
gtcgtgaatt cctgggtac cgatgtgatg agggctgatg tgttctcgt gcggtggga    600
gaacataacc tgtacagcac taacgactcg tcagacccaa tcgacttcgc cgtgaacgagc    660
gtgaagcacc atgaaaactt cgtgctggcc acctacctca acgacatcgc gatcctgaag    720
ctgaacgaca ctgtcacctt cacacacaag attaagccga ttgcctccc ttacgagtc    780
ctgcgctatg aggacctcgc tatgcgcaac cccttctgtg ccggatgggg cactaccgcg    840
ttcaacggac cgagcagcgc tgctctgaga gaagtgaac tcccaatctg gggtcacgag    900
ccgtgccgcc aagcgtacga gaaagacctg aacatcacca acgtgtatat gtgcgogggc    960
tacgccgacg gtgaaagga cgcattgccg ggcgattctg gcgccccat gatgctgect   1020
gacaagtcgc ggaacttcta cctggtcgga atcgtgtcgt tcggaagaa atgcgccctg   1080
cccgctttc ccggagtgtg caccaaggtc accgaatttt tggattggat tgcgctgaac   1140
atgggtctaa                                     1149

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<210> SEQ ID NO 8

<211> LENGTH: 354

<212> TYPE: PRT

<213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 8

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Ser Ser Leu Gly Arg Gln Arg Arg Gln Phe Val Phe Pro Asp Asp Glu
1          5          10          15
Glu Ser Cys Ser Asn Arg Phe Thr Asn Asp Gly Ile Cys Lys Asp Val
20          25          30
Leu Asn Cys Arg Asp Leu Leu Gln Lys Asn Asp Tyr Asn Leu Leu Lys
35          40          45
Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro Lys Val Cys Cys Pro
50          55          60
Lys Gln Ser Ile Val Asn Pro Ile Thr Glu Ala Pro Pro Lys Thr Thr
65          70          75          80
Thr Thr Glu Arg Pro Pro Ile Arg Ile Pro Ser Asn Leu Pro Lys Gln
85          90          95
Cys Gly Asn Arg Asn Ile Thr Thr Thr Arg Ile Ile Gly Gly Gln Glu
100         105         110
Ala Thr Pro Gly Ala Trp Pro Trp Met Ala Ala Val Tyr Ile Lys Gln
115         120         125
Gly Gly Ile Arg Ser Val Gln Cys Gly Gly Ala Leu Val Thr Asn Arg

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130	135	140
His Val Ile Thr Ala Ser His Cys Val Val Asn Ser Leu Gly Thr Asp 145	150	155
Val Met Arg Ala Asp Val Phe Ser Val Arg Leu Gly Glu His Asn Leu 165	170	175
Tyr Ser Thr Asn Asp Ser Ser Asp Pro Ile Asp Phe Ala Val Thr Ser 180	185	190
Val Lys His His Glu Asn Phe Val Leu Ala Thr Tyr Leu Asn Asp Ile 195	200	205
Ala Ile Leu Lys Leu Asn Asp Thr Val Thr Phe Thr His Lys Ile Lys 210	215	220
Pro Ile Cys Leu Pro Tyr Glu Ser Leu Arg Tyr Glu Asp Leu Ala Met 225	230	235
Arg Asn Pro Phe Val Ala Gly Trp Gly Thr Thr Ala Phe Asn Gly Pro 245	250	255
Ser Ser Ala Val Leu Arg Glu Val Gln Leu Pro Ile Trp Gly His Glu 260	265	270
Pro Cys Arg Gln Ala Tyr Glu Lys Asp Leu Asn Ile Thr Asn Val Tyr 275	280	285
Met Cys Ala Gly Tyr Ala Asp Gly Gly Lys Asp Ala Cys Gln Gly Asp 290	295	300
Ser Gly Gly Pro Met Met Leu Pro Asp Lys Ser Gly Asn Phe Tyr Leu 305	310	315
Val Gly Ile Val Ser Phe Gly Lys Lys Cys Ala Leu Pro Gly Phe Pro 325	330	335
Gly Val Tyr Thr Lys Val Thr Glu Phe Leu Asp Trp Ile Ala Val Asn 340	345	350
Met Val		

<210> SEQ ID NO 9
 <211> LENGTH: 382
 <212> TYPE: PRT
 <213> ORGANISM: Limulus polyphemus

<400> SEQUENCE: 9

Met Gly Ile Leu Pro Ser Pro Gly Met Pro Ala Leu Leu Ser Leu Val 1	5	10	15
Ser Leu Leu Ser Val Leu Leu Met Gly Cys Val Ala Ser Ser Leu Gly 20	25	30	
Arg Gln Arg Arg Gln Phe Val Phe Pro Asp Asp Glu Glu Ser Cys Ser 35	40	45	
Asn Arg Phe Thr Asn Asp Gly Ile Cys Lys Asp Val Leu Asn Cys Arg 50	55	60	
Asp Leu Leu Gln Lys Asn Asp Tyr Asn Leu Leu Lys Glu Ser Ile Cys 65	70	75	80
Gly Phe Glu Gly Ile Thr Pro Lys Val Cys Cys Pro Lys Gln Ser Ile 85	90	95	
Val Asn Pro Ile Thr Glu Ala Pro Pro Lys Thr Thr Thr Thr Glu Arg 100	105	110	
Pro Pro Ile Arg Ile Pro Ser Asn Leu Pro Lys Gln Cys Gly Asn Arg 115	120	125	
Asn Ile Thr Thr Thr Arg Ile Ile Gly Gly Gln Glu Ala Thr Pro Gly			

-continued

130	135	140
Ala Trp Pro Trp Met Ala Ala Val Tyr Ile Lys Gln Gly Gly Ile Arg 145 150 155 160		
Ser Val Gln Cys Gly Gly Ala Leu Val Thr Asn Arg His Val Ile Thr 165 170 175		
Ala Ser His Cys Val Val Asn Ser Leu Gly Thr Asp Val Met Arg Ala 180 185 190		
Asp Val Phe Ser Val Arg Leu Gly Glu His Asn Leu Tyr Ser Thr Asn 195 200 205		
Asp Ser Ser Asp Pro Ile Asp Phe Ala Val Thr Ser Val Lys His His 210 215 220		
Glu Asn Phe Val Leu Ala Thr Tyr Leu Asn Asp Ile Ala Ile Leu Lys 225 230 235 240		
Leu Asn Asp Thr Val Thr Phe Thr His Lys Ile Lys Pro Ile Cys Leu 245 250 255		
Pro Tyr Glu Ser Leu Arg Tyr Glu Asp Leu Ala Met Arg Asn Pro Phe 260 265 270		
Val Ala Gly Trp Gly Thr Thr Ala Phe Asn Gly Pro Ser Ser Ala Val 275 280 285		
Leu Arg Glu Val Gln Leu Pro Ile Trp Gly His Glu Pro Cys Arg Gln 290 295 300		
Ala Tyr Glu Lys Asp Leu Asn Ile Thr Asn Val Tyr Met Cys Ala Gly 305 310 315 320		
Tyr Ala Asp Gly Gly Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro 325 330 335		
Met Met Leu Pro Asp Lys Ser Gly Asn Phe Tyr Leu Val Gly Ile Val 340 345 350		
Ser Phe Gly Lys Lys Cys Ala Leu Pro Gly Phe Pro Gly Val Tyr Thr 355 360 365		
Lys Val Thr Glu Phe Leu Asp Trp Ile Ala Val Asn Met Val 370 375 380		

<210> SEQ ID NO 10

<211> LENGTH: 3051

<212> TYPE: DNA

<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 10

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atgaagttcc tcgtcaacgt cgcctcgtg tttatggtgg tgtatattag ctacatctat    60
gctcgtggcg tcgatctggg cctgtgcgat gagaccgct tcgaatgtaa gtgcggcgac    120
cccggtctatg tgttcaacgt ccccatgaag cagtgcacct acttctaccg ctggcgcccc    180
tactgtaagc cttgcgatga cctggaagct aaagatatct gccctaagta taagcgctgc    240
caagaatgta aggtggtct ggattcctgc gtcacctgtc cccctaacaa gtatggcacc    300
tggtgttccg gtgaatgccg gtgcaaaaac ggcggcatct gcgatcagcg tactggcgct    360
tgcacctgcc gtgatcgcta cgaggcgccc cactgtgaaa ttctgaaagg ctgtcctctg    420
ctgccttccg atagccaagt gcaggaggtg cgtaaccccc ctgacaaccc tcagaccatc    480
gattatagct gctcccccg cttcaagctg aaggcgctgg cccgtattag ctgtctgccc    540
aacggtcagt ggagctcctt tccccccaag tgcacccgtg aatgtgcaa ggtctcctcc    600
cccgaacacg gcaaggtcaa cgctccctcc ggcaacatga tcgaaggcgc caccctccgt    660

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ttttcctgcg	actccoctta	ctacetcate	ggccaggaga	ccttgaactg	tcagggcaat	720
ggccagtggg	gcggtcaaat	ccctcagtgc	aagaagctgg	tgttctgtcc	tgacctcgat	780
cccgtgaacc	acgctgagca	ccaagtgaag	atcggcgtgg	aacaaaagta	cggtcagttc	840
cctcagggca	ctgaggtcac	ttacacttgc	tccggcaact	acttctgat	gggcttcaac	900
acctgaaat	gtaacccga	cggttcctgg	tccggttccc	agccctctg	cgtgaaagtc	960
gctgaccgtg	aagtcgactg	tgacagcaaa	gccgtggact	tcctggacga	cgtgggcgag	1020
cctgtgcgta	ttactgttcc	cgtgggtgt	agcctgactg	ccggtaccgt	gtgggggtact	1080
gctatctacc	acgagctctc	cagcgtgtgc	cgcgctgcta	tccacgtgg	caagctgcct	1140
aacagcggcg	gtgctgtcca	cgtggtgaac	aacggtcctt	actccgactt	cctcggtagc	1200
gacctgaacg	gtatcaaaa	cgaggaactg	aagtcctctg	cccgtagctt	tcgcttcgac	1260
tacgtgtcct	cctccaccgc	tggccgtage	ggctgtcctg	atggctggtt	tgaggtgga	1320
gagaattgtg	tgtactgtac	ctccaagcag	cgtgcttggg	agcgtgctca	gggtgtctgt	1380
accaatatgg	ccgccgtct	ggctgtcttc	gataaggatc	tcctcccttc	ctccctcacc	1440
gagactctgc	gcggtaaagg	cctgaccact	acctggatcg	gtctccaccg	cctggacgct	1500
gagaagcctt	tcgtctggga	gctcatggac	cgcagcaacg	tggctgtgaa	cgacaacctg	1560
acctctctgg	ccagcggcga	gcctggcaat	gaaaccaact	gcgtgtacct	ggacatccgt	1620
gatcagctcc	agcccggtg	gaagaccaag	agctgcttcc	agccctccag	ctttgcttgc	1680
atgatggatc	tctccgaccg	caacaaggct	aatgtgatg	atcccgccc	cctggagaa	1740
ggccatgcca	ctctgcatgg	ccagagcacc	gacggtttct	acgtgggttc	ctccatccgc	1800
tactcctgcg	aagtgtctca	ctacctgtcc	ggcaccgaga	ccgtgacctg	tactaccaac	1860
ggtaacttga	gcgctcctaa	accccgctgt	atcaaggatg	ttacctgtca	gaacctcct	1920
gtccccagct	acggtccgt	ggaaatcaag	ccccccagcc	gtactaatag	catcagccgt	1980
gtcgggtccc	ctttcctgcg	tctgcctcgt	ctgcccctgc	ctctcgctcg	cgtgctaag	2040
cctcccccta	agccccgttc	cagccagcct	agcactgtgg	atctcgctag	caaagtga	2100
ctccccgagg	gtactatcgc	tgtgggtccc	cgcgccattt	acacctgcga	gagccgctac	2160
tacgagctcc	tgggcagcca	gggtcgtcgt	tgcgacagca	acggtaactg	gagcggctgc	2220
cctgccagct	gcattcccg	gtgcgggtgc	agcgactccc	ctcgagccc	tttcatctgg	2280
aacggcaaca	gcaccgagat	cgttcagtgg	ccttggcaag	ctggcattag	ccgctggctc	2340
gctgatcaca	acatgtgggt	cctccaatgt	ggcggcagcc	tgctgaacga	gaagtggatc	2400
gtgactgcgc	ctcactgcgt	gaacctacgc	gccaccgctg	agatcatcga	ccctccca	2460
tttaagatct	acctgggcaa	gtactaccgc	gacgatagcc	gcgacgacga	ttatgtgcaa	2520
gtccgcgaag	ctctcgaaat	tcacgtgaac	cctaactacg	atcccggtaa	cctgaacttc	2580
gacattgccc	tgatccagct	gaagacccct	gtgacctca	ccactcgcgt	ccagcctatc	2640
tgctcccca	ccgacattac	cactcgcgaa	catctgaaag	aaggtaccct	ggcgcgtcg	2700
accggctggg	gcctcaacga	aaacaacact	tacagcgaga	tgatccagca	ggctgtgctg	2760
cctgtcgtgg	ctgctagcac	ctgtgaggaa	ggctacaaag	aggccgatct	ccccctgacc	2820
gtcaccgaga	acatgttttg	cgtcgggtac	aaaaagggcc	gctacgacgc	ctgcagcggt	2880
gatagcggtg	gcctctctct	gttcgccgat	gattcccgtg	ccgaacgtcg	ctgggtgctc	2940

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gagggcattg tgcctgggg tagcccttcc ggctgtggca aggctaacca atacggcggc 3000
 ttcaccaagg tgaacgtgtt cctgtcctgg attcgtcagt tcataaatg a 3051

<210> SEQ ID NO 11
 <211> LENGTH: 994
 <212> TYPE: PRT
 <213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 11

Arg Gly Val Asp Leu Gly Leu Cys Asp Glu Thr Arg Phe Glu Cys Lys
 1 5 10 15
 Cys Gly Asp Pro Gly Tyr Val Phe Asn Val Pro Met Lys Gln Cys Thr
 20 25 30
 Tyr Phe Tyr Arg Trp Arg Pro Tyr Cys Lys Pro Cys Asp Asp Leu Glu
 35 40 45
 Ala Lys Asp Ile Cys Pro Lys Tyr Lys Arg Cys Gln Glu Cys Lys Ala
 50 55 60
 Gly Leu Asp Ser Cys Val Thr Cys Pro Pro Asn Lys Tyr Gly Thr Trp
 65 70 75 80
 Cys Ser Gly Glu Cys Gln Cys Lys Asn Gly Gly Ile Cys Asp Gln Arg
 85 90 95
 Thr Gly Ala Cys Thr Cys Arg Asp Arg Tyr Glu Gly Ala His Cys Glu
 100 105 110
 Ile Leu Lys Gly Cys Pro Leu Leu Pro Ser Asp Ser Gln Val Gln Glu
 115 120 125
 Val Arg Asn Pro Pro Asp Asn Pro Gln Thr Ile Asp Tyr Ser Cys Ser
 130 135 140
 Pro Gly Phe Lys Leu Lys Gly Val Ala Arg Ile Ser Cys Leu Pro Asn
 145 150 155 160
 Gly Gln Trp Ser Ser Phe Pro Pro Lys Cys Ile Arg Glu Cys Ala Lys
 165 170 175
 Val Ser Ser Pro Glu His Gly Lys Val Asn Ala Pro Ser Gly Asn Met
 180 185 190
 Ile Glu Gly Ala Thr Leu Arg Phe Ser Cys Asp Ser Pro Tyr Tyr Leu
 195 200 205
 Ile Gly Gln Glu Thr Leu Thr Cys Gln Gly Asn Gly Gln Trp Ser Gly
 210 215 220
 Gln Ile Pro Gln Cys Lys Lys Leu Val Phe Cys Pro Asp Leu Asp Pro
 225 230 235 240
 Val Asn His Ala Glu His Gln Val Lys Ile Gly Val Glu Gln Lys Tyr
 245 250 255
 Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn
 260 265 270
 Tyr Phe Leu Met Gly Phe Asn Thr Leu Lys Cys Asn Pro Asp Gly Ser
 275 280 285
 Trp Ser Gly Ser Gln Pro Ser Cys Val Lys Val Ala Asp Arg Glu Val
 290 295 300
 Asp Cys Asp Ser Lys Ala Val Asp Phe Leu Asp Asp Val Gly Glu Pro
 305 310 315 320
 Val Arg Ile His Cys Pro Ala Gly Cys Ser Leu Thr Ala Gly Thr Val
 325 330 335

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Trp	Gly	Thr	Ala	Ile	Tyr	His	Glu	Leu	Ser	Ser	Val	Cys	Arg	Ala	Ala
			340					345					350		
Ile	His	Ala	Gly	Lys	Leu	Pro	Asn	Ser	Gly	Gly	Ala	Val	His	Val	Val
		355					360					365			
Asn	Asn	Gly	Pro	Tyr	Ser	Asp	Phe	Leu	Gly	Ser	Asp	Leu	Asn	Gly	Ile
		370				375					380				
Lys	Ser	Glu	Glu	Leu	Lys	Ser	Leu	Ala	Arg	Ser	Phe	Arg	Phe	Asp	Tyr
385					390					395					400
Val	Ser	Ser	Ser	Thr	Ala	Gly	Arg	Ser	Gly	Cys	Pro	Asp	Gly	Trp	Phe
				405					410					415	
Glu	Val	Glu	Glu	Asn	Cys	Val	Tyr	Val	Thr	Ser	Lys	Gln	Arg	Ala	Trp
				420				425					430		
Glu	Arg	Ala	Gln	Gly	Val	Cys	Thr	Asn	Met	Ala	Ala	Arg	Leu	Ala	Val
		435					440					445			
Leu	Asp	Lys	Asp	Leu	Ile	Pro	Ser	Ser	Leu	Thr	Glu	Thr	Leu	Arg	Gly
		450				455					460				
Lys	Gly	Leu	Thr	Thr	Thr	Trp	Ile	Gly	Leu	His	Arg	Leu	Asp	Ala	Glu
465					470				475						480
Lys	Pro	Phe	Val	Trp	Glu	Leu	Met	Asp	Arg	Ser	Asn	Val	Val	Leu	Asn
			485					490						495	
Asp	Asn	Leu	Thr	Phe	Trp	Ala	Ser	Gly	Glu	Pro	Gly	Asn	Glu	Thr	Asn
			500					505					510		
Cys	Val	Tyr	Leu	Asp	Ile	Arg	Asp	Gln	Leu	Gln	Pro	Val	Trp	Lys	Thr
		515					520					525			
Lys	Ser	Cys	Phe	Gln	Pro	Ser	Ser	Phe	Ala	Cys	Met	Met	Asp	Leu	Ser
		530				535					540				
Asp	Arg	Asn	Lys	Ala	Lys	Cys	Asp	Asp	Pro	Gly	Pro	Leu	Glu	Asn	Gly
545					550				555						560
His	Ala	Thr	Leu	His	Gly	Gln	Ser	Ile	Asp	Gly	Phe	Tyr	Ala	Gly	Ser
			565					570						575	
Ser	Ile	Arg	Tyr	Ser	Cys	Glu	Val	Leu	His	Tyr	Leu	Ser	Gly	Thr	Glu
			580					585					590		
Thr	Val	Thr	Cys	Thr	Thr	Asn	Gly	Thr	Trp	Ser	Ala	Pro	Lys	Pro	Arg
			595				600					605			
Cys	Ile	Lys	Val	Ile	Thr	Cys	Gln	Asn	Pro	Pro	Val	Pro	Ser	Tyr	Gly
		610				615					620				
Ser	Val	Glu	Ile	Lys	Pro	Pro	Ser	Arg	Thr	Asn	Ser	Ile	Ser	Arg	Val
625					630				635						640
Gly	Ser	Pro	Phe	Leu	Arg	Leu	Pro	Arg	Leu	Pro	Leu	Pro	Leu	Ala	Arg
			645					650						655	
Ala	Ala	Lys	Pro	Pro	Pro	Lys	Pro	Arg	Ser	Ser	Gln	Pro	Ser	Thr	Val
			660					665					670		
Asp	Leu	Ala	Ser	Lys	Val	Lys	Leu	Pro	Glu	Gly	His	Tyr	Arg	Val	Gly
		675					680					685			
Ser	Arg	Ala	Ile	Tyr	Thr	Cys	Glu	Ser	Arg	Tyr	Tyr	Glu	Leu	Leu	Gly
		690				695					700				
Ser	Gln	Gly	Arg	Arg	Cys	Asp	Ser	Asn	Gly	Asn	Trp	Ser	Gly	Arg	Pro
705					710				715						720
Ala	Ser	Cys	Ile	Pro	Val	Cys	Gly	Arg	Ser	Asp	Ser	Pro	Arg	Ser	Pro
			725					730					735		
Phe	Ile	Trp	Asn	Gly	Asn	Ser	Thr	Glu	Ile	Gly	Gln	Trp	Pro	Trp	Gln

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740	745	750
Ala Gly Ile Ser Arg Trp Leu Ala Asp His Asn Met Trp Phe Leu Gln 755	760	765
Cys Gly Gly Ser Leu Leu Asn Glu Lys Trp Ile Val Thr Ala Ala His 770	775	780
Cys Val Thr Tyr Ser Ala Thr Ala Glu Ile Ile Asp Pro Ser Gln Phe 785	790	795
Lys Ile Tyr Leu Gly Lys Tyr Tyr Arg Asp Asp Ser Arg Asp Asp Asp 805	810	815
Tyr Val Gln Val Arg Glu Ala Leu Glu Ile His Val Asn Pro Asn Tyr 820	825	830
Asp Pro Gly Asn Leu Asn Phe Asp Ile Ala Leu Ile Gln Leu Lys Thr 835	840	845
Pro Val Thr Leu Thr Thr Arg Val Gln Pro Ile Cys Leu Pro Thr Asp 850	855	860
Ile Thr Thr Arg Glu His Leu Lys Glu Gly Thr Leu Ala Val Val Thr 865	870	875
Gly Trp Gly Leu Asn Glu Asn Asn Thr Tyr Ser Glu Met Ile Gln Gln 885	890	895
Ala Val Leu Pro Val Val Ala Ala Ser Thr Cys Glu Glu Gly Tyr Lys 900	905	910
Glu Ala Asp Leu Pro Leu Thr Val Thr Glu Asn Met Phe Cys Ala Gly 915	920	925
Tyr Lys Lys Gly Arg Tyr Asp Ala Cys Ser Gly Asp Ser Gly Gly Pro 930	935	940
Leu Val Phe Ala Asp Asp Ser Arg Thr Glu Arg Arg Trp Val Leu Glu 945	950	955
Gly Ile Val Ser Trp Gly Ser Pro Ser Gly Cys Gly Lys Ala Asn Gln 965	970	975
Tyr Gly Gly Phe Thr Lys Val Asn Val Phe Leu Ser Trp Ile Arg Gln 980	985	990

Phe Ile

<210> SEQ ID NO 12

<211> LENGTH: 1015

<212> TYPE: PRT

<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 12

Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile 1	5	10	15
--	---	----	----

Ser Tyr Ile Tyr Ala Arg Gly Val Asp Leu Gly Leu Cys Asp Glu Thr 20	25	30
---	----	----

Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val Phe Asn Val Pro 35	40	45
---	----	----

Met Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro Tyr Cys Lys Pro 50	55	60
---	----	----

Cys Asp Asp Leu Glu Ala Lys Asp Ile Cys Pro Lys Tyr Lys Arg Cys 65	70	75	80
---	----	----	----

Gln Glu Cys Lys Ala Gly Leu Asp Ser Cys Val Thr Cys Pro Pro Asn 85	90	95
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Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys Lys Asn Gly Gly

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100						105						110					
Ile	Cys	Asp	Gln	Arg	Thr	Gly	Ala	Cys	Thr	Cys	Arg	Asp	Arg	Tyr	Glu		
115						120						125					
Gly	Ala	His	Cys	Glu	Ile	Leu	Lys	Gly	Cys	Pro	Leu	Leu	Pro	Ser	Asp		
130						135						140					
Ser	Gln	Val	Gln	Glu	Val	Arg	Asn	Pro	Pro	Asp	Asn	Pro	Gln	Thr	Ile		
145						150						155					
Asp	Tyr	Ser	Cys	Ser	Pro	Gly	Phe	Lys	Leu	Lys	Gly	Val	Ala	Arg	Ile		
165						170						175					
Ser	Cys	Leu	Pro	Asn	Gly	Gln	Trp	Ser	Ser	Phe	Pro	Pro	Lys	Cys	Ile		
180						185						190					
Arg	Glu	Cys	Ala	Lys	Val	Ser	Ser	Pro	Glu	His	Gly	Lys	Val	Asn	Ala		
195						200						205					
Pro	Ser	Gly	Asn	Met	Ile	Glu	Gly	Ala	Thr	Leu	Arg	Phe	Ser	Cys	Asp		
210						215						220					
Ser	Pro	Tyr	Tyr	Leu	Ile	Gly	Gln	Glu	Thr	Leu	Thr	Cys	Gln	Gly	Asn		
225						230						235					
Gly	Gln	Trp	Ser	Gly	Gln	Ile	Pro	Gln	Cys	Lys	Lys	Leu	Val	Phe	Cys		
245						250						255					
Pro	Asp	Leu	Asp	Pro	Val	Asn	His	Ala	Glu	His	Gln	Val	Lys	Ile	Gly		
260						265						270					
Val	Glu	Gln	Lys	Tyr	Gly	Gln	Phe	Pro	Gln	Gly	Thr	Glu	Val	Thr	Tyr		
275						280						285					
Thr	Cys	Ser	Gly	Asn	Tyr	Phe	Leu	Met	Gly	Phe	Asn	Thr	Leu	Lys	Cys		
290						295						300					
Asn	Pro	Asp	Gly	Ser	Trp	Ser	Gly	Ser	Gln	Pro	Ser	Cys	Val	Lys	Val		
305						310						315					
Ala	Asp	Arg	Glu	Val	Asp	Cys	Asp	Ser	Lys	Ala	Val	Asp	Phe	Leu	Asp		
325						330						335					
Asp	Val	Gly	Glu	Pro	Val	Arg	Ile	His	Cys	Pro	Ala	Gly	Cys	Ser	Leu		
340						345						350					
Thr	Ala	Gly	Thr	Val	Trp	Gly	Thr	Ala	Ile	Tyr	His	Glu	Leu	Ser	Ser		
355						360						365					
Val	Cys	Arg	Ala	Ala	Ile	His	Ala	Gly	Lys	Leu	Pro	Asn	Ser	Gly	Gly		
370						375						380					
Ala	Val	His	Val	Val	Asn	Asn	Gly	Pro	Tyr	Ser	Asp	Phe	Leu	Gly	Ser		
385						390						395					
Asp	Leu	Asn	Gly	Ile	Lys	Ser	Glu	Glu	Leu	Lys	Ser	Leu	Ala	Arg	Ser		
405						410						415					
Phe	Arg	Phe	Asp	Tyr	Val	Ser	Ser	Ser	Thr	Ala	Gly	Arg	Ser	Gly	Cys		
420						425						430					
Pro	Asp	Gly	Trp	Phe	Glu	Val	Glu	Glu	Asn	Cys	Val	Tyr	Val	Thr	Ser		
435						440						445					
Lys	Gln	Arg	Ala	Trp	Glu	Arg	Ala	Gln	Gly	Val	Cys	Thr	Asn	Met	Ala		
450						455						460					
Ala	Arg	Leu	Ala	Val	Leu	Asp	Lys	Asp	Leu	Ile	Pro	Ser	Ser	Leu	Thr		
465						470						475					
Glu	Thr	Leu	Arg	Gly	Lys	Gly	Leu	Thr	Thr	Thr	Trp	Ile	Gly	Leu	His		
485						490						495					
Arg	Leu	Asp	Ala	Glu	Lys	Pro	Phe	Val	Trp	Glu	Leu	Met	Asp	Arg	Ser		
500						505						510					

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Asn Val	Val	Leu	Asn	Asp	Asn	Leu	Thr	Phe	Trp	Ala	Ser	Gly	Glu	Pro
	515					520					525			
Gly Asn	Glu	Thr	Asn	Cys	Val	Tyr	Leu	Asp	Ile	Arg	Asp	Gln	Leu	Gln
	530				535					540				
Pro Val	Trp	Lys	Thr	Lys	Ser	Cys	Phe	Gln	Pro	Ser	Ser	Phe	Ala	Cys
	545			550					555					560
Met Met	Asp	Leu	Ser	Asp	Arg	Asn	Lys	Ala	Lys	Cys	Asp	Asp	Pro	Gly
		565						570					575	
Pro Leu	Glu	Asn	Gly	His	Ala	Thr	Leu	His	Gly	Gln	Ser	Ile	Asp	Gly
		580					585					590		
Phe Tyr	Ala	Gly	Ser	Ser	Ile	Arg	Tyr	Ser	Cys	Glu	Val	Leu	His	Tyr
	595					600					605			
Leu Ser	Gly	Thr	Glu	Thr	Val	Thr	Cys	Thr	Thr	Asn	Gly	Thr	Trp	Ser
	610				615					620				
Ala Pro	Lys	Pro	Arg	Cys	Ile	Lys	Val	Ile	Thr	Cys	Gln	Asn	Pro	Pro
	625			630					635					640
Val Pro	Ser	Tyr	Gly	Ser	Val	Glu	Ile	Lys	Pro	Pro	Ser	Arg	Thr	Asn
		645					650						655	
Ser Ile	Ser	Arg	Val	Gly	Ser	Pro	Phe	Leu	Arg	Leu	Pro	Arg	Leu	Pro
		660					665					670		
Leu Pro	Leu	Ala	Arg	Ala	Ala	Lys	Pro	Pro	Pro	Lys	Pro	Arg	Ser	Ser
	675					680					685			
Gln Pro	Ser	Thr	Val	Asp	Leu	Ala	Ser	Lys	Val	Lys	Leu	Pro	Glu	Gly
	690				695					700				
His Tyr	Arg	Val	Gly	Ser	Arg	Ala	Ile	Tyr	Thr	Cys	Glu	Ser	Arg	Tyr
	705			710					715					720
Tyr Glu	Leu	Leu	Gly	Ser	Gln	Gly	Arg	Arg	Cys	Asp	Ser	Asn	Gly	Asn
		725					730						735	
Trp Ser	Gly	Arg	Pro	Ala	Ser	Cys	Ile	Pro	Val	Cys	Gly	Arg	Ser	Asp
		740					745					750		
Ser Pro	Arg	Ser	Pro	Phe	Ile	Trp	Asn	Gly	Asn	Ser	Thr	Glu	Ile	Gly
		755				760						765		
Gln Trp	Pro	Trp	Gln	Ala	Gly	Ile	Ser	Arg	Trp	Leu	Ala	Asp	His	Asn
	770				775					780				
Met Trp	Phe	Leu	Gln	Cys	Gly	Gly	Ser	Leu	Leu	Asn	Glu	Lys	Trp	Ile
	785			790					795					800
Val Thr	Ala	Ala	His	Cys	Val	Thr	Tyr	Ser	Ala	Thr	Ala	Glu	Ile	Ile
		805						810					815	
Asp Pro	Ser	Gln	Phe	Lys	Ile	Tyr	Leu	Gly	Lys	Tyr	Tyr	Arg	Asp	Asp
		820					825					830		
Ser Arg	Asp	Asp	Asp	Tyr	Val	Gln	Val	Arg	Glu	Ala	Leu	Glu	Ile	His
		835				840						845		
Val Asn	Pro	Asn	Tyr	Asp	Pro	Gly	Asn	Leu	Asn	Phe	Asp	Ile	Ala	Leu
	850				855					860				
Ile Gln	Leu	Lys	Thr	Pro	Val	Thr	Leu	Thr	Thr	Arg	Val	Gln	Pro	Ile
	865			870						875				880
Cys Leu	Pro	Thr	Asp	Ile	Thr	Thr	Arg	Glu	His	Leu	Lys	Glu	Gly	Thr
		885						890					895	
Leu Ala	Val	Val	Thr	Gly	Trp	Gly	Leu	Asn	Glu	Asn	Asn	Thr	Tyr	Ser
		900					905						910	

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Glu	Met	Ile	Gln	Gln	Ala	Val	Leu	Pro	Val	Val	Ala	Ala	Ser	Thr	Cys
	915						920						925		
Glu	Glu	Gly	Tyr	Lys	Glu	Ala	Asp	Leu	Pro	Leu	Thr	Val	Thr	Glu	Asn
	930					935					940				
Met	Phe	Cys	Ala	Gly	Tyr	Lys	Lys	Gly	Arg	Tyr	Asp	Ala	Cys	Ser	Gly
945					950				955						960
Asp	Ser	Gly	Gly	Pro	Leu	Val	Phe	Ala	Asp	Asp	Ser	Arg	Thr	Glu	Arg
				965					970					975	
Arg	Trp	Val	Leu	Glu	Gly	Ile	Val	Ser	Trp	Gly	Ser	Pro	Ser	Gly	Cys
		980						985					990		
Gly	Lys	Ala	Asn	Gln	Tyr	Gly	Gly	Phe	Thr	Lys	Val	Asn	Val	Phe	Leu
	995						1000					1005			
Ser	Trp	Ile	Arg	Gln	Phe	Ile									
	1010					1015									

<210> SEQ ID NO 13

<211> LENGTH: 1200

<212> TYPE: DNA

<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 13

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cgctgtgtgg ggggtgctgtt ccccaagacc cggaacgaca acgaatgcac tgccaggggt    120
ggattgaagg ggctctgcaa gagcctgata gactgcccgt cgggtgctgc caccctgaag    180
gactcctttc ctgtcgtgtg ctcttggaac ggcagattcc agccaattgt gtgctgtccg    240
gatgcaattg cccccccacc tgtgaccacc accgcccgtga ccgtgatetc taccaaggaa    300
cccaagctgc ctgcgctcca catcagcggg tgtgggaagc ggaagggtcaa gattgacatt    360
accactgtgg gacgtcagc atcaccgatt ctgccccga tctccacccc gcaaaactcc    420
actggtggca ggggcatcat tgccgggtga gtggaagcga agatcggagc ctggccttgg    480
atggcggcgg tgtttgtgaa aaacttcgga atcgggcggg tccactgcgc tgggtcgatc    540
atctccaaca agtacatcct gtcgccgca catgccttcc tgatcggagg ccggaagtgt    600
acccccacga gactggcctg cagagtcgga gggcattaca tcaagcgcgg acaggaatac    660
cccgtaaggg acgtgataat ccacccccac tacgtggaaa aggagaacta caatgacatc    720
gccattatcg agctgaagga ggagctgaac ttaccgatc ttgtgaaccc catctgcctc    780
cctgaccccc agacagtgc cgaaccactc aaagaccgca tcgtgactgc agctggatgg    840
ggcgatctgg acttcagcgg accgagaagc caggctcctgc gggaggtgtc aatcccggtg    900
gtgccagtcg acaaatgcga tcaggcctac gagaagctga acactccctc cctcaaaaac    960
gggatcacta acaacttcct gtgcgcggga cttgaagaag gcggaaggga cgctgtcag    1020
ggcgattccg gcggacctct gatgctcgtg aacaatactc ggtggattgt cgtcggcgtg    1080
gtgtccttcg gacataagtg cgcgaggaa ggctaccctg gcgtctatcc ccgctgggcc    1140
tcctacctgg actggattgc caaggtcacc aactcgctcg accatgcctg gactaaactga    1200

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<210> SEQ ID NO 14

<211> LENGTH: 377

<212> TYPE: PRT

<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 14

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Val	Gly	Val	Leu	Phe	Pro	Lys	Thr	Arg	Asn	Asp	Asn	Glu	Cys	Thr	Ala	1	5	10	15
Arg	Gly	Gly	Leu	Lys	Gly	Ser	Cys	Lys	Ser	Leu	Ile	Asp	Cys	Pro	Ser	20	25	30	
Val	Leu	Ala	Thr	Leu	Lys	Asp	Ser	Phe	Pro	Val	Val	Cys	Ser	Trp	Asn	35	40	45	
Gly	Arg	Phe	Gln	Pro	Ile	Val	Cys	Cys	Pro	Asp	Ala	Ile	Ala	Pro	Pro	50	55	60	
Pro	Val	Thr	Thr	Thr	Ala	Val	Thr	Val	Ile	Ser	Thr	Lys	Glu	Pro	Lys	65	70	75	80
Leu	Pro	Arg	Leu	His	Ile	Ser	Gly	Cys	Gly	Lys	Arg	Lys	Val	Lys	Ile	85	90	95	
Asp	Ile	Thr	Thr	Val	Gly	Arg	Ser	Gly	Ser	Pro	Ile	Leu	Pro	Pro	Ile	100	105	110	
Ser	Thr	Pro	Gln	Asn	Ser	Thr	Gly	Gly	Arg	Gly	Ile	Ile	Ala	Gly	Gly	115	120	125	
Val	Glu	Ala	Lys	Ile	Gly	Ala	Trp	Pro	Trp	Met	Ala	Ala	Val	Phe	Val	130	135	140	
Lys	Asn	Phe	Gly	Ile	Gly	Arg	Phe	His	Cys	Ala	Gly	Ser	Ile	Ile	Ser	145	150	155	160
Asn	Lys	Tyr	Ile	Leu	Ser	Ala	Ala	His	Ala	Phe	Leu	Ile	Gly	Gly	Arg	165	170	175	
Lys	Leu	Thr	Pro	Thr	Arg	Leu	Ala	Val	Arg	Val	Gly	Gly	His	Tyr	Ile	180	185	190	
Lys	Arg	Gly	Gln	Glu	Tyr	Pro	Val	Lys	Asp	Val	Ile	Ile	His	Pro	His	195	200	205	
Tyr	Val	Glu	Lys	Glu	Asn	Tyr	Asn	Asp	Ile	Ala	Ile	Ile	Glu	Leu	Lys	210	215	220	
Glu	Glu	Leu	Asn	Phe	Thr	Asp	Leu	Val	Asn	Pro	Ile	Cys	Leu	Pro	Asp	225	230	235	240
Pro	Glu	Thr	Val	Thr	Asp	Pro	Leu	Lys	Asp	Arg	Ile	Val	Thr	Ala	Ala	245	250	255	
Gly	Trp	Gly	Asp	Leu	Asp	Phe	Ser	Gly	Pro	Arg	Ser	Gln	Val	Leu	Arg	260	265	270	
Glu	Val	Ser	Ile	Pro	Val	Val	Pro	Val	Asp	Lys	Cys	Asp	Gln	Ala	Tyr	275	280	285	
Glu	Lys	Leu	Asn	Thr	Pro	Ser	Leu	Lys	Asn	Gly	Ile	Thr	Asn	Asn	Phe	290	295	300	
Leu	Cys	Ala	Gly	Leu	Glu	Glu	Gly	Gly	Lys	Asp	Ala	Cys	Gln	Gly	Asp	305	310	315	320
Ser	Gly	Gly	Pro	Leu	Met	Leu	Val	Asn	Asn	Thr	Arg	Trp	Ile	Val	Val	325	330	335	
Gly	Val	Val	Ser	Phe	Gly	His	Lys	Cys	Ala	Glu	Glu	Gly	Tyr	Pro	Gly	340	345	350	
Val	Tyr	Ser	Arg	Val	Ala	Ser	Tyr	Leu	Asp	Trp	Ile	Ala	Lys	Val	Thr	355	360	365	
Asn	Ser	Leu	Asp	His	Ala	Val	Thr	Asn	370	375									

<210> SEQ ID NO 15

<211> LENGTH: 399

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<212> TYPE: PRT
<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 15

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp
1          5          10          15

Phe Pro Gly Ser Arg Cys Val Gly Val Leu Phe Pro Lys Thr Arg Asn
20          25          30

Asp Asn Glu Cys Thr Ala Arg Gly Gly Leu Lys Gly Ser Cys Lys Ser
35          40          45

Leu Ile Asp Cys Pro Ser Val Leu Ala Thr Leu Lys Asp Ser Phe Pro
50          55          60

Val Val Cys Ser Trp Asn Gly Arg Phe Gln Pro Ile Val Cys Cys Pro
65          70          75          80

Asp Ala Ile Ala Pro Pro Val Thr Thr Thr Ala Val Thr Val Ile
85          90          95

Ser Thr Lys Glu Pro Lys Leu Pro Arg Leu His Ile Ser Gly Cys Gly
100         105         110

Lys Arg Lys Val Lys Ile Asp Ile Thr Thr Val Gly Arg Ser Gly Ser
115         120         125

Pro Ile Leu Pro Pro Ile Ser Thr Pro Gln Asn Ser Thr Gly Gly Arg
130         135         140

Gly Ile Ile Ala Gly Gly Val Glu Ala Lys Ile Gly Ala Trp Pro Trp
145         150         155         160

Met Ala Ala Val Phe Val Lys Asn Phe Gly Ile Gly Arg Phe His Cys
165         170         175

Ala Gly Ser Ile Ile Ser Asn Lys Tyr Ile Leu Ser Ala Ala His Ala
180         185         190

Phe Leu Ile Gly Gly Arg Lys Leu Thr Pro Thr Arg Leu Ala Val Arg
195         200         205

Val Gly Gly His Tyr Ile Lys Arg Gly Gln Glu Tyr Pro Val Lys Asp
210         215         220

Val Ile Ile His Pro His Tyr Val Glu Lys Glu Asn Tyr Asn Asp Ile
225         230         235         240

Ala Ile Ile Glu Leu Lys Glu Glu Leu Asn Phe Thr Asp Leu Val Asn
245         250         255

Pro Ile Cys Leu Pro Asp Pro Glu Thr Val Thr Asp Pro Leu Lys Asp
260         265         270

Arg Ile Val Thr Ala Ala Gly Trp Gly Asp Leu Asp Phe Ser Gly Pro
275         280         285

Arg Ser Gln Val Leu Arg Glu Val Ser Ile Pro Val Val Pro Val Asp
290         295         300

Lys Cys Asp Gln Ala Tyr Glu Lys Leu Asn Thr Pro Ser Leu Lys Asn
305         310         315         320

Gly Ile Thr Asn Asn Phe Leu Cys Ala Gly Leu Glu Glu Gly Gly Lys
325         330         335

Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Leu Val Asn Asn
340         345         350

Thr Arg Trp Ile Val Val Gly Val Val Ser Phe Gly His Lys Cys Ala
355         360         365

Glu Glu Gly Tyr Pro Gly Val Tyr Ser Arg Val Ala Ser Tyr Leu Asp
370         375         380

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Trp Ile Ala Lys Val Thr Asn Ser Leu Asp His Ala Val Thr Asn
385 390 395

<210> SEQ ID NO 16
<211> LENGTH: 1128
<212> TYPE: DNA
<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 16

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tgctccaccc tctcgagaca gcggcgccag ttgtgttcc ctgatgaaga ggaactgtgt 120
tccaaccggt tcaccgaaga gggaacttgc aagaactgac tggactgcgc catcctgctt 180
caaaagaacg actacaacct tctcaaggag tcaatctgac gtttcgaagg gattacccca 240
aaagtctgct gcccgaagtc ctgcacgtg atctcaagca cccaggcacc cctgaaact 300
accactactg agcggccccc gaagcaaatt ccccgaaact tgccggaagt ctgcggcatc 360
cacaacacca ccaccactag gatcatcggg gggagagaag cccctatcgc cgctggccc 420
tggatgacgc ctgtgtacat taagcagggc ggtatccgga gcgtgcagtg cggaggagct 480
ctggtcacca atcgccacgt gatcacagcg tcacactgac tctgaactc ggccgggtacc 540
gacgtgatgc ccgccgacgt gttctctgtg cgggtggggg aacataacct ttactcgacc 600
gatgatgact ccaacccaat cgatttcgcc gtgacgtcgc tgaagcacca tgagcacttc 660
gtgctggcca cctacctgaa cgacatagca attctcactc tgaacgacac cgtgactttt 720
actgatcgga tcaggccaat ctgtctgcgc taccgcaagc tcagatacga cgacctggcc 780
atgcgcaagc cgttcattac ggggtgggga actaccgcct tcaacggacc ttctccgcc 840
gtgtgcggg aagtgcagct gccgatctgg gagcacgaag cctgtagaca agcctacgaa 900
aaggaccta acattaccaa cgtgtatatg tgtgcggat tcgcggatgg cggcaaagac 960
gcatgccagg gagactccgg cggcccgatg atgtgcctg tgaaaaccgg agagttctac 1020
ctcatcggca tcgtcagctt cgggaagaaa tgcgccctgc ccgattccc cggagtgtac 1080
actaaggtea ccgagttcct cgactggatt gccgaacaca tgggtgtaa 1128
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<210> SEQ ID NO 17
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 17

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Ser Thr Leu Ser Arg Gln Arg Arg Gln Phe Val Phe Pro Asp Glu Glu
1 5 10 15
Glu Leu Cys Ser Asn Arg Phe Thr Glu Glu Gly Thr Cys Lys Asn Val
20 25 30
Leu Asp Cys Arg Ile Leu Leu Gln Lys Asn Asp Tyr Asn Leu Leu Lys
35 40 45
Glu Ser Ile Cys Gly Phe Glu Gly Ile Thr Pro Lys Val Cys Cys Pro
50 55 60
Lys Ser Ser His Val Ile Ser Ser Thr Gln Ala Pro Pro Glu Thr Thr
65 70 75 80
Thr Thr Glu Arg Pro Pro Lys Gln Ile Pro Pro Asn Leu Pro Glu Val
85 90 95
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Cys	Gly	Ile	His	Asn	Thr	Thr	Thr	Thr	Arg	Ile	Ile	Gly	Gly	Arg	Glu
			100					105					110		
Ala	Pro	Ile	Gly	Ala	Trp	Pro	Trp	Met	Thr	Ala	Val	Tyr	Ile	Lys	Gln
		115					120					125			
Gly	Gly	Ile	Arg	Ser	Val	Gln	Cys	Gly	Gly	Ala	Leu	Val	Thr	Asn	Arg
		130				135					140				
His	Val	Ile	Thr	Ala	Ser	His	Cys	Val	Val	Asn	Ser	Ala	Gly	Thr	Asp
145					150					155					160
Val	Met	Pro	Ala	Asp	Val	Phe	Ser	Val	Arg	Leu	Gly	Glu	His	Asn	Leu
				165					170					175	
Tyr	Ser	Thr	Asp	Asp	Ser	Asn	Pro	Ile	Asp	Phe	Ala	Val	Thr	Ser	
			180				185					190			
Val	Lys	His	His	Glu	His	Phe	Val	Leu	Ala	Thr	Tyr	Leu	Asn	Asp	Ile
		195					200					205			
Ala	Ile	Leu	Thr	Leu	Asn	Asp	Thr	Val	Thr	Phe	Thr	Asp	Arg	Ile	Arg
		210				215					220				
Pro	Ile	Cys	Leu	Pro	Tyr	Arg	Lys	Leu	Arg	Tyr	Asp	Asp	Leu	Ala	Met
225					230					235					240
Arg	Lys	Pro	Phe	Ile	Thr	Gly	Trp	Gly	Thr	Thr	Ala	Phe	Asn	Gly	Pro
			245						250					255	
Ser	Ser	Ala	Val	Leu	Arg	Glu	Val	Gln	Leu	Pro	Ile	Trp	Glu	His	Glu
			260					265					270		
Ala	Cys	Arg	Gln	Ala	Tyr	Glu	Lys	Asp	Leu	Asn	Ile	Thr	Asn	Val	Tyr
		275					280					285			
Met	Cys	Ala	Gly	Phe	Ala	Asp	Gly	Gly	Lys	Asp	Ala	Cys	Gln	Gly	Asp
		290				295					300				
Ser	Gly	Gly	Pro	Met	Met	Leu	Pro	Val	Lys	Thr	Gly	Glu	Phe	Tyr	Leu
305					310					315					320
Ile	Gly	Ile	Val	Ser	Phe	Gly	Lys	Lys	Cys	Ala	Leu	Pro	Gly	Phe	Pro
			325						330					335	
Gly	Val	Tyr	Thr	Lys	Val	Thr	Glu	Phe	Leu	Asp	Trp	Ile	Ala	Glu	His
			340				345					350			
Met	Val														

<210> SEQ ID NO 18

<211> LENGTH: 375

<212> TYPE: PRT

<213> ORGANISM: Tachypleus tridentatus

<400> SEQUENCE: 18

Met	Leu	Val	Asn	Asn	Val	Phe	Ser	Leu	Leu	Cys	Phe	Pro	Leu	Leu	Met
1			5					10						15	
Ser	Val	Val	Arg	Cys	Ser	Thr	Leu	Ser	Arg	Gln	Arg	Arg	Gln	Phe	Val
			20					25					30		
Phe	Pro	Asp	Glu	Glu	Glu	Leu	Cys	Ser	Asn	Arg	Phe	Thr	Glu	Glu	Gly
		35				40						45			
Thr	Cys	Lys	Asn	Val	Leu	Asp	Cys	Arg	Ile	Leu	Leu	Gln	Lys	Asn	Asp
		50				55				60					
Tyr	Asn	Leu	Leu	Lys	Glu	Ser	Ile	Cys	Gly	Phe	Glu	Gly	Ile	Thr	Pro
65				70					75					80	
Lys	Val	Cys	Cys	Pro	Lys	Ser	Ser	His	Val	Ile	Ser	Ser	Thr	Gln	Ala
			85					90						95	

```
<210> SEQ ID NO 19
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 19
```

Ile Glu Gly Arg
1

- 1-36. (canceled)
37. A method of preparing an amebocyte lysate composition comprising:
- (a) providing a recombinant factor C, a recombinant factor B, and a recombinant pro-clotting enzyme; and
 - (b) combining the recombinant factor C, the recombinant factor B, and the recombinant pro-clotting enzyme to formulate the amebocyte lysate composition;
- wherein at least one of the recombinant factor B, recombinant factor C, or recombinant pro-clotting enzyme is made by expression in a host cell that has been genetically modified to prevent the addition of sialic acid to the recombinant factor B, the recombinant factor C, and/or the recombinant pro-clotting enzyme.
38. The method of claim 37, wherein the recombinant factor C is made by expression in a host cell that has been genetically modified to prevent the addition of sialic acid to the recombinant factor C.
39. The method of claim 37, wherein the amebocyte lysate composition remains stable in a salt solution.
40. The method of claim 37, wherein the recombinant factor C, the recombinant factor B, and the recombinant pro-clotting enzyme each comprise an amino acid sequence of a *Limulus polyphemus*, *Tachypleus gigas*, *Tachypleus tridentatus*, or *Carcinoscorpius rotundicauda* factor C, factor B, or pro-clotting enzyme.
41. The method of claim 40, wherein the recombinant factor C comprises the amino acid sequence of SEQ ID NO:2, the recombinant factor B comprises the amino acid sequence of SEQ ID NO: 5, and the recombinant pro-clotting enzyme comprises the amino acid sequence of SEQ ID NO: 8.
42. The method of claim 37, wherein the host cell is a mammalian host cell.
43. The method of claim 37, wherein the host cell is a HEK293 cell.
44. The method of claim 37, wherein the host cell lacks N-acetylglucosaminyltransferase I.
45. The method of claim 37, wherein step (b) is performed in a vial or on a cartridge for use in bacterial endotoxin testing.
46. A composition comprising a recombinant factor C, a recombinant factor B, and a recombinant pro-clotting enzyme, wherein at least one of the recombinant factor B, the recombinant factor C, or the recombinant pro-clotting enzyme lacks sialic acid.
47. The composition of claim 46, wherein the recombinant factor C lacks sialic acid.
48. The composition of claim 46, wherein said composition remains stable in a salt solution.
49. The composition of claim 46, wherein the recombinant factor C, recombinant factor B, and recombinant pro-clotting enzyme each comprise the amino acid sequence of a *Limulus polyphemus*, *Tachypleus gigas*, *Tachypleus tridentatus*, or *Carcinoscorpius rotundicauda* factor C, factor B, or pro-clotting enzyme.
50. The method of claim 49, wherein the recombinant factor C comprises the amino acid sequence of SEQ ID NO:2, the recombinant factor B comprises the amino acid sequence of SEQ ID NO: 5, and the recombinant pro-clotting enzyme comprises the amino acid sequence of SEQ ID NO: 8.
51. The composition of claim 46, wherein the composition is contained within a vial or on a cartridge for use in bacterial endotoxin testing.
52. A method of producing a recombinant factor C protein comprising:
- (a) culturing a host cell transfected with a vector comprising a nucleic acid sequence encoding a factor C protein to obtain a cell culture; and
 - (b) purifying the factor C protein from the cell culture;
- wherein the host cell is a host cell that has been genetically modified to prevent the addition of sialic acid to the factor C protein.
53. The method of claim 52, wherein the recombinant factor C protein remains stable in a salt solution.
54. The method of claim 52, wherein the recombinant factor C comprises the amino acid sequence of a *Limulus polyphemus*, *Tachypleus gigas*, *Tachypleus tridentatus*, or *Carcinoscorpius rotundicauda* factor C.
55. The method of claim 52, wherein the nucleic acid encodes the amino acid sequence of SEQ ID NO:2.
56. The method of claim 52, wherein the host cell is a mammalian host cell.
57. The method of claim 52, wherein the host cell is a HEK293 cell.
58. The method of claim 52, wherein the host cell lacks N-acetylglucosaminyltransferase I.
59. A purified recombinant factor C protein produced by the method of claim 52.
60. A host cell comprising a nucleic acid encoding a factor C protein, wherein the factor C protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:2, wherein the host cell has been genetically modified to prevent addition of sialic acid to the factor C protein.
61. The host cell of claim 60, wherein the host cell is a mammalian host cell.
62. The host cell of claim 60, wherein the host cell is a HEK293 cell.
63. The host cell of claim 60, wherein the host cell lacks N-acetylglucosaminyltransferase I.
64. The host cell of claim 60, wherein the nucleic acid encodes a factor C protein comprising the amino acid sequence of SEQ ID NO:2.
65. A kit for bacterial endotoxin testing comprising the recombinant factor C protein of claim 59, a recombinant factor B, and a recombinant pro-clotting enzyme.

* * * * *