47	Foreign Reference	WO2007081387_part_5_QC.pdf	23872461 80f1ddac3cae7d69c9062baad3d3478ccb318224	no	20
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48	Foreign Reference	WO2007081387_part_6_QC.pdf	21911487 f2148c10735028609f3b170666b94fee6a0d6558	no	29
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49	Foreign Reference	WO2007084192_QC.pdf	2539489 843557947aa7aefd817b53e0dbdbcf4de700a432	no	44
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50	Foreign Reference	WO2007098148_part_1_QC.pdf	23275305 2b5c13b111558136697 37eccb9023cb1651ab6f4	no	25
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51	Foreign Reference	WO2007098148_part_2_QC.pdf	23648393 53a876134672cc2bb92b6a76798351c79b6baead	no	28
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52	Foreign Reference	WO2007098148_part_3_QC.pdf	11472694 892a29325fc3ecdd8e72fc5bc9573657f0f63fa7	no	29
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53	Foreign Reference	WO2007114947_QC.pdf	5563586 3327f29951c85eab3af54ef921813b978d234fb4	no	78
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54	Foreign Reference	WO2008048371_QC.pdf	7246665 04183bb7bd24ff40960ae6892b40247a609d5950	no	116
Warnings:					
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55	Foreign Reference	WO2009029073_part_1_QC.pdf	23595838 09024ec401535b2e0be0d8c8a86aec2c596aa12e	no	27
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56	Foreign Reference	WO2009029073_part_2_QC.pdf	24045589 3c739c70d81fac13f5cc701551f489e492346cf1	no	36
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Information:					
57	Foreign Reference	WO2009029073_part_3_QC.pdf	4781512 062c9120be9d9426da44b085098917fbe404d3ec	no	10
Warnings:					
Information:					
58	Foreign Reference	WO2009156747A3_QC.pdf	234489 4be84c738f3bd5e25b83f914201f7ac3ddfc74c7	no	6
Warnings:					
Information:					
59	Foreign Reference	WO2010039180A2_QC.pdf	4954262 cfdfe42c6d1f7fb86af0f312b6c97bd161d7e949	no	98
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60	Foreign Reference	WO2011032155A2_QC.pdf	7363760 218f2813a3ffcb789870c21cb1af78a72568c0f1	no	112
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		Total Files Size (in bytes):	1076867114		

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

Electronic Acknowledgement Receipt

EFS ID:	22157455
Application Number:	14111326
International Application Number:	
Confirmation Number:	3274
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Eileen MacKenzie
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018US01
Receipt Date:	28-APR-2015
Filing Date:	24-JUN-2014
Time Stamp:	19:58:48
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Johnson_et_al_AnalChem_1997_v69_p4641_QC.pdf	12449717 f37d504bcb80a36507b7253f007124136e699c18	no	8

Warnings:	
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2	Non Patent Literature	Joos_Presentation_2013_43pgs_QC.pdf	7313506 c0858711d3fe97b62f6a8dd202a29af733046ddb	no	43
Warnings:					
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3	Non Patent Literature	Ke_et_al_PLoS_One_2013_v8_pE69813_QC.pdf	338523 b38bc2ee8c4b4d33c8e61ced033479030ab3c748	no	5
Warnings:					
Information:					
4	Non Patent Literature	Kiss_et_al_Anal_Chem_2008_v80_p8975_QC.pdf	1182515 ffe1754a1fd532e54b65e7c2f81366e6b37d30bd	no	17
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5	Non Patent Literature	Kremsky_et_al_NucleicAcidsRes_1987_v15_p2891_QC.pdf	12759876 014e9b2ec61f874836ac107996d17cedc47267bd	no	19
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6	Non Patent Literature	Kuang_et_al_AnalChem_2004_v76_p2902_QC.pdf	13092704 79f8cf5dd7ac073aca0745a802127c36a2a055a3	no	8
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7	Non Patent Literature	Kuang_et_al_AnalBiochem_2005_v345_p320_QC.pdf	8796458 61fc99904fa20539bdd8635bb91e6cf4c2f4a2e7	no	6
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8	Non Patent Literature	Kuang_et_al_AnalChem_2004_v76_p6282_QC.pdf	7415780 c163d6c2d71c062f17ea1f61f3e6dd183bf24895	no	5
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9	Non Patent Literature	LaFratta_et_al_ChemRev_2008_v108_p614_part_1_QC.pdf	22446756 90e11a5725fd98d96c00ff894348ee8f80924970	no	10
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10	Non Patent Literature	LaFratta_et_al_ChemRev_2008_v108_p614_part_2_QC.pdf	23692860 1356a0bf2b12849802c5197152ab8d5cadbf273d	no	10
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11	Non Patent Literature	LaFratta_et_al_ChemRev_2008_v108_p614_part_3_QC.pdf	7541548 743b09bbb6207a11a43488da4aae24f46ceb1a97	no	4
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12	Non Patent Literature	Lee_et_al_AnalBiochem_2000_v282_p142_QC.pdf	9775265 e8d09f5972c7a111f64c6fa7bbc79191984fe4cc	no	5
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13	Non Patent Literature	Li_et_al_JAmChemSoc_2008_v130_p12622_QC.pdf	3399863 55542a9206f151dac7734059826efab30c13e93f	no	2
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14	Non Patent Literature	Li_et_al_Neurobiol_Aging_2009_v30_p1091_QC.pdf	1876230 77f3962d958da1e20d579ccdf605870637d4d5c2	no	8
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15	Non Patent Literature	Li_et_al_AnalChem_2004_v76_p4446_QC.pdf	9907948 acf5d51b3d94f15feeea9617f645dddefd3bcbcb	no	6
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16	Non Patent Literature	Lu_et_al_Science_1998_v282_p1877_QC.pdf	13606889 83ab1ecd3172f15912c26c2435befe7e2f20ab51	no	7
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17	Non Patent Literature	Luo_et_al_PNAS_2007_v104_p12610_QC.pdf	12953429 57c3cf21f79fb55961d2553363f3cf02fe0bb645	no	6
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18	Non Patent Literature	Melin_et_al_AnnuRevBiophysBiomolStruct_2007_v36_p213_QC.pdf	24645385 781aed4e0fee2ade9f67a5de990cc5a1546de464	no	21
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19	Non Patent Literature	Michael_et_al_AnalBiochem_1999_v273_p168_QC.pdf	24157600 90865b495bb139438d295ccffb2fd29195951e87	no	11
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20	Non Patent Literature	Michael_et_al_AnalChem_1998_v70_p1242_QC.pdf	10792463 d8660fe316fc7b0f960d5a4713f6f8c0b7d25d37	no	7
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21	Non Patent Literature	Mondello_et_al_Abstract_26th_Annual_Cong_Eur_Soc_Intens_Care_Med_2013_2pgs.pdf	54832 5184f46d8429c3f7f593c42737bd33964afb8688	no	2
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22	Non Patent Literature	Mondello_et_al_Scientific_Reports_2014_v4_6pgs.pdf	750702 e3057e263da6bd319ab2930ed281b59647986ecf	no	6
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23	Non Patent Literature	Monk_et_al_JAmChemSoc_2004_v126_p11416_QC.pdf	3835586 0a53aff85c537d68475bb4915dca577d11edadba4	no	2
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24	Non Patent Literature	Monk_et_al_AnalBioanalChem_2004_v379_p931_QC.pdf	26152239 984a990a58fbe03f4ee53512fb9cd258d4952c4c	no	15
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25	Non Patent Literature	Monk_et_al_JMaterChem_2005_v15_p4361_QC.pdf	9334957 6fa504ace9536ee080c1db8657df2e268384d44c	no	6
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26	Non Patent Literature	Morrison_et_al_Nucl_Acids_Res_2006_v34_pe123_QC.pdf	679427 b2f40b013fac43a5ca4864a5eaadb4586a87eabe	no	9
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27	Non Patent Literature	Munkholm_et_al_AnalChem_1986_v58_p1427_QC.pdf	6052200 e2a1ecba0efa2caaa5e396f5f28642628c7fdbf8	no	4
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28	Non Patent Literature	Nagai_et_al_BiosensBioelectron_2001_v16_p1015_QC.pdf	7629290 e98a0bc77600bc8623b67f24c25d98fa015d59db	no	5
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29	Non Patent Literature	Nalefski_et_al_ClinChem_2006_v52_p2172_QC.pdf	5936108 5ceb458bab8df073e859ef4b41871df679e642d6	no	4
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30	Non Patent Literature	Nam_et_al_Science_2003_v301_p1884_QC.pdf	5938236 2dedaacb902e14a2035acaaf39028c10b12f020d	no	3
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31	Non Patent Literature	Neselius_et_al_Brain_Inj_2013_v27_p425.pdf	565627 7992f36fda2874d8d607cb43af2735deb125ce62	no	9
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32	Non Patent Literature	Niemeyer_et_al_NucAcidsRes_2003_v31_pe90_QC.pdf	180834 bdbbffbd630a43e7910ab2d5e780a22cac59c0d1	no	7
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33	Non Patent Literature	Okrongly_Abst_Pres_ISE_Intl_Conf_2010_24pgs_QC.pdf	5064833 e39f7c73f20087e72f960d67d7aefa77c5bac861	no	24
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34	Non Patent Literature	Panova_et_al_AnalChem_1997_v69_p1635_QC.pdf	15045211 438f7d6fdb172c8a13439963aa311d9025999801	no	7
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36	Non Patent Literature	Pantano_et_al_AnalChem_1995_v67_p481A_part_2_QC.pdf	18610662 3bf521296580ac687667873 3c984ab5c1e91b714	no	3
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37	Non Patent Literature	Pantano_et_al_AnalChem_1995_v67_p481A_part_3_QC.pdf	5697629 bfa0dec054d248f576aa3f229ce61aea66c5b588	no	1
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38	Non Patent Literature	Pantano_et_al_ChemMater_1996_v8_p2832_QC.pdf	8233630 a9ff781b4024067574868d9cff2ef15462ff9171	no	4
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39	Non Patent Literature	Pantano_et_al_RevSciInstrum_1997_v68_p1357_QC.pdf	5950536 a1559d411120dfcb13292092f367b2adc9631b22	no	3
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40	Non Patent Literature	Peterson_et_al_AnalChem_1980_v52_p864_QC.pdf	11512184 e203d04f4cbe83941da8785673a920f737fa52af	no	6
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41	Non Patent Literature	Prabhakar_et_al_J_Immunol_Methods_2002_v260_p207_QC.pdf	1567628 d2d777d127da3979d99e8c2e17d9bec870f8c481	no	12
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42	Non Patent Literature	Qiu_et_al_ClinChem_2007_v53_p2010_QC.pdf	5758555 4b0fdabc95eaaec9043b449363d54cd7955b6bca	no	3
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43	Non Patent Literature	Randall_et_al_Abstract_Poster_Neuroscience_Conf_2013_2pgs_QC.pdf	1346144 967bacf39f7f37a7c39287b6671ccc6b05ea5b27	no	2
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44	Non Patent Literature	Randall_et_al_Abstract_Poster_Neuroscience_Conf_2011_2pgs_QC.pdf	3806601 da3eb6f795130595785c53aa25a73c805ebf1536	no	2
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45	Non Patent Literature	Randall_et_al_Resuscitation_2013_v84_p351_Epub_6pgs_QC.pdf	314434 efd188b4321a60de5e5e2c4ca79dfb06dbf1e8c0	no	6
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46	Non Patent Literature	Randle_et_al_BiochemBiophysResCommun_2004_v324_P504_QC.pdf	10548759 588766244b24d06e859534f83a32a9d7206fba29	no	7
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47	Non Patent Literature	Rissin_et_al_Oak_Ridge_Conf_Poster_7_Abstract_2011_2pgs_QC.pdf	798009 76687a0122f2ed58c4cbdfab802b9f1082259e42	no	2
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48	Non Patent Literature	Rissin_et_al_NanoLett_2006_v6_p520_QC.pdf	6653669 c7d0755f894bd88698096beaaf860922f4b60114	no	4
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49	Non Patent Literature	Rissin_et_al_JAmChemSoc_2006_v128_6286_QC.pdf	3447909 1e070236421cf88344a9e20d8ccaeff2aa99b7ad	no	2
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50	Non Patent Literature	Rissin_et_al_JAmChemSoc_2008_v130_p5349_QC.pdf	7397184 818df5f893d3a35a809b6b0392a75d9a209e9dd9	no	5
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51	Non Patent Literature	Rissin_et_al_AnalChimActa_2006_v564_p34_QC.pdf	8735060 7a04d5b51c34532d95dc8031a8c255caeaa449bc	no	6
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52	Non Patent Literature	Rissin_et_al_Lab_Chip_2013_v13_p2902_QC.pdf	1379364 4cd4d1613f93094a2f80631dc6cca43cf3b2b677	no	19
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53	Non Patent Literature	Rissin_et_al_Anal_Chem_2010_p2279.pdf	3280896 7d4e0103959f8d1add53537ada8fe32b35dd2c31	no	7
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54	Non Patent Literature	Rissin_et_al_NatBiotechnol_2010_v28_p595_Suppl_Pgs_QC.pdf	944942 094dcda3123c32d8ef5ddddc3d5589f50589c9b6	no	10
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55	Non Patent Literature	Rissin_et_al_AttomolarDetectionProteinsInSerum_ConfPoster_2010_QC_pdf.pdf	3626849 e79893454ba512d8f997e9a599232eb9e13cc92b	no	1
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56	Non Patent Literature	Roeffaers_et_al_PNAS_2007_v104_p12603_QC.pdf	15756657 80660e0e6150130dcb0f9f4e16997829a658e9e7	no	7
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57	Non Patent Literature	Rondelez_et_al_Nature_2005_v433_p773_QC.pdf	8346573 e0444fd5fbcffe415bdfb5120254714c65b2b69f	no	5
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58	Non Patent Literature	Rondelez_et_al_NatBiotechnol_2005_v23_p361_QC.pdf	12132816 4ad688cde409f9e6cc7501efea60aec6ac847755	no	5
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59	Non Patent Literature	Rotman_PNAS_1961_v47_p1981_QC.pdf	11970253 d1d59b2bcc32d727f0b48dc5c5004b90c3a270a0	no	11
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60	Non Patent Literature	Schauer_et_al_JAmChemSoc_2001_v123_p9443_QC.pdf	3238028 790d4802ddb2bf9ae85afefba1bd7b6b03fd9d83	no	2
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		Total Files Size (in bytes):	495462649		

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<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

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

Electronic Acknowledgement Receipt

EFS ID:	22157875
Application Number:	14111326
International Application Number:	
Confirmation Number:	3274
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Eileen MacKenzie
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018US01
Receipt Date:	28-APR-2015
Filing Date:	24-JUN-2014
Time Stamp:	20:00:55
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Schmidinger_et_al_ChemBioChem_2006_v7_527_QC.pdf	11906800 defe20ba4e374e0cabebb5a1fa69060ee08a7c50	no	8

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2	Non Patent Literature	Schweitzer_et_al_PNAS_2000_v97_p10113_QC.pdf	12498134 d9deac0461198f4daa5115c7f87ac5979c9925a2	no	7
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3	Non Patent Literature	Seydack_BiosensBioelectron_2005_v20_p2454_part_1_QC.pdf	23211264 e5f35d21671ad4c84ab1456086f007cdbd6e8c47	no	11
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5	Non Patent Literature	Shen_et_al_Mutat_Res_2005_v573_p70.pdf	2693136 4d70e5ec7700cb03176daeeb379cf88c1a9dcb02	no	13
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6	Non Patent Literature	Shepard_et_al_AnalChem_2005_v77_p319_QC.pdf	13268020 265aee11acee8f2f63d07cf7d2dd1e50a282cfea	no	8
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7	Non Patent Literature	Song_et_al_EmergInfectDis_2005_v11_p1629_QC.pdf	9339837 9a84c2421ac3b2fcc88635427f8b0864467e31ec	no	4
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8	Non Patent Literature	Shahim_et_al_JAMA_Neurol_2014_v71_p684_Epub_9pgs_QC.pdf	252132 bf388d940b721acb595671d91c25afd722322dc8	no	9
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9	Non Patent Literature	Shiiya_et_al_Ann_Thorac_Surg_2004_v77_p2034_QC.pdf	100568 eeff561cf0c791a67e937fd5ccdadc37b463d00c	no	5
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10	Non Patent Literature	Song_et_al_Abstract_Poster_Oakridge_Conf_2013_2pgs_QC.PDF	775196 c6861a88bf2f8f999074811bf63ec3c8dc15bab3	no	2
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11	Non Patent Literature	Song_et_al_Anal_Chem_2013_v85_p1932_QC.pdf	2839113 77518ff6281f2f89e8ab2a1a3fef7a098b2ffa4e	no	22
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12	Non Patent Literature	Song_et_al_AnalChem_2006_v78_p1023_QC.pdf	19549162 23a69349f009ec4d87bbdd74b98c59aa05e21b71	no	12
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13	Non Patent Literature	Song_et_al_J_Immunol_Meth_2011_v372_p177_QC.pdf	299194 47802d974f5387b3c16240732ccdb4995072593d	no	10
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14	Non Patent Literature	Soukka_et_al_clinChem_2001_v47_p1269_QC.pdf	17065920 e66af52ef1b9bf645189b30282fd80f387487fad	no	10
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15	Non Patent Literature	Stamou_et_al_AngewChemInt Ed_2003_v42_p5580_QC.pdf	6881500 797e06ee3eb34ece0acc7723018338aa74f269bb	no	4
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16	Non Patent Literature	Steemers_et_al_MikrochimActa_1999_v131_p99_QC.pdf	8707183 ba1164f142291df86bfa9f0810f03cb53c8c05e8	no	7
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18	Non Patent Literature	Stitzel_et_al_AnalChem_2001_v73_p5266_QC.pdf	9239845 0544229b5fa69eb36991f0b1e9219952540899b3	no	6
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19	Non Patent Literature	Subbaraman_Tech_Rev_Website_06-03-2010_2pgs.pdf	595876 bc3d5f9a7dc57ab56fd22e228f19f96e096e3e0e	no	2
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20	Non Patent Literature	Sun_et_al_PNAS_2006_v103_p18727_QC.pdf	950262 ff6df32782095515bd053281257b080f34a5a70b	no	6
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21	Non Patent Literature	Sykes_et_al_Biotechniques_1992_v13_p444_QC.pdf	11035685 810812d110adfdf6426ffb31e94855d4b1252feb	no	6
Warnings:					
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22	Non Patent Literature	Szunerits_et_al_AnalChem_2002_v74_p886_QC.pdf	14406049 11451929b3641e73dbf84c12baa808e22945428f	no	9
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23	Non Patent Literature	Szunerits_et_al_AnalChem_2002_v74_p1718_QC.pdf	8833852 bc1235bf84c01ac60815adff1954237b6228e1c5	no	6
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24	Non Patent Literature	Szunerits_et_al_AnalChem_2003_v75_p4382_QC.pdf	12705800 7b0334081ad2c6b0c4790fa116b7a924281fdb53	no	7
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25	Non Patent Literature	Szunerits_et_al_Chemophyschem_2003_v17_p186_QC.pdf	11303239 59a709715c9367c987fe40f1517e7a79b6d0245f	no	14
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26	Non Patent Literature	Szurdoki_et_al_AnalBiochem_2001_v291_p219_QC.pdf	19565994 3bad2f78e4dfba696b1ab25a300af11a4d5b46dd	no	10
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27	Non Patent Literature	Tam_et_al_ApplPhysLett_2004_v84_p4289_QC.pdf	6195393 6baa33e2b77fc7062f55cecd199ceb28d52cdd8f2	no	3
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Information:					
28	Non Patent Literature	Tam_et_al_Talanta_2005_v67_p498_QC.pdf	8587131 f5392090390dad692d178f6f253dc011ee80b502	no	5
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Information:					

29	Non Patent Literature	Tam_et_al_ApplPhysLett_2006_v89_p194101_QC.pdf	6027868 fd244d822535e0af0a0a6699b2b03f6b0569254e	no	3
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Information:					
30	Non Patent Literature	Tan_et_al_AnalChem_1997_v69_p4242_QC.pdf	12957966 dfa78e59d074000cfcc6a95eddd429e1f162f87f	no	7
Warnings:					
Information:					
31	Non Patent Literature	Tanen_et_al_Poster_Abstract_AAPS_Mtg_2014_2pgs_QC.pdf	886648 646d1ee2712edeb616f22f772b635c82afdb0df7	no	2
Warnings:					
Information:					
32	Non Patent Literature	Taylor_et_al_AnalBiochem_2000_v278_p132_QC.pdf	19920605 c9d66250d984f5e8c4b3c12c69d21680ced2e0ef	no	11
Warnings:					
Information:					
33	Non Patent Literature	Taylor_et_al_J_Biol_Chem_1999_v274_p31217_QC.pdf	168014 e7fc079f9bb6bcc487ea771d2314decf943123be	no	6
Warnings:					
Information:					
34	Non Patent Literature	Tessler_et_al_AnalChem_2009_v81_p7141_QC.pdf	13957097 390f80362b1364e78faa9a51e0935fd9413757c0	no	8
Warnings:					
Information:					
35	Non Patent Literature	Timmerman_Xconomy_Website_Published_01-19-2010_4pgs.pdf	744715 355db1dcb5eb2a0d6722eb7755acec7f1fabd5f0	no	4
Warnings:					
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36	Non Patent Literature	Todd_et_al_ClinChem_2007_v53_p1990_QC.pdf	8455330 e14c2b3a4f751319fdfb6f351d16f5aa6a60cc86	no	6
Warnings:					
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37	Non Patent Literature	Tromberg_et_al_AnalChem_1988_v60_p1901_QC.pdf	16170784 fc1b01790afa396d743aa9d6af47ece48c71b6d9	no	8
Warnings:					
Information:					

38	Non Patent Literature	Ueberfeld_et_al_AnalChem_2004_v76_p947_QC.pdf	6879698 d4995163e92a99ae0d9648e063ec88e9adbed935	no	6
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39	Non Patent Literature	Vo-Dinh_et_al_ApplSpectrosc_1990_v44_p128_QC.pdf	8808897 4948a59769e7f160666568658e24f15e11e1bb1a3	no	5
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Information:					
40	Non Patent Literature	Vogelstein_et_al_Proc_Natl_Acad_Sci_1999_v96_p9236_QC.pdf	879557 c6f1b5f5bacd4efa3b366be11a7b0268eb41f322	no	6
Warnings:					
Information:					
41	Non Patent Literature	Walt_et_al_RadiatRes_2001_v156_p442_QC.pdf	6815856 cda3d770edeeec6da510c3beaefdf02ee4d6ff35	no	3
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42	Non Patent Literature	Walt_et_al_AnnNYAcadSci_2007_v1098_p389_QC.pdf	15120372 29ee904192f937df426e8cd699380139507 13d64	no	12
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43	Non Patent Literature	Walt_et_al_BiosensBioelectron_1998_v13_p697_QC.pdf	4511629 5d0c8c7e6684fdce982c95277a35a4619163ec30	no	3
Warnings:					
Information:					
44	Non Patent Literature	Walt_Ultrasensitive_detection_proteins_Pittcon_PowerPoint_2010_32pgs.pdf	4838585 1e8543826428ba5963dd9cc0e0b41121594ab0dd	no	33
Warnings:					
Information:					
45	Non Patent Literature	Walt_oemagazine_2005_p19_QC.pdf	7319869 93fc38c6c11498003ba17b8c94d3894678ffac7d	no	3
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Information:					
46	Non Patent Literature	Walt_Biotechniques_2006_v41_p529_QC.pdf	7907611 20f7431cd5530e3c3ef3e918921f0719e2daeff6	no	4
Warnings:					
Information:					

47	Non Patent Literature	Walt_AccChemRes_1998_v31_p267_QC.pdf	20110269 834a09e69516c2bcafec04973373b343ec29beb6	no	12
Warnings:					
Information:					
48	Non Patent Literature	Walt_CurrOpinChemBiol_2002_v6_p689_QC.pdf	12680584 cc982ec68d514206a0d19b37adcd7b879d9fac20	no	7
Warnings:					
Information:					
49	Non Patent Literature	Walt_Anal_Chem_2013_v85_p1258.pdf	2447030 e1eb47821af4883b1205e3b50e41a164a7c144b3	no	6
Warnings:					
Information:					
50	Non Patent Literature	Walt_Science_2000_v287_p451_QC.pdf	5156144 4242b4c36eb3b58ccd53f42bab01d295fd1528d2	no	3
Warnings:					
Information:					
51	Non Patent Literature	Wang_et_al_AnalChimActa_2007_v590_p104_QC.pdf	7495413 7bca475086acf331d5d20c58f992cd0eb40e84a7	no	6
Warnings:					
Information:					
52	Non Patent Literature	Warren_et_al_PNAS_2006_v21_p17807_QC.pdf	1522080 32e23f32b317bd0c75454fcaebfc3c1f8d020c25	no	6
Warnings:					
Information:					
53	Non Patent Literature	Whitaker_et_al_AnalBiochem_2007_v360_p63_QC.pdf	18419352 5ba777570098 90a41eb57162458c26f85c82ebb6	no	12
Warnings:					
Information:					
54	Non Patent Literature	Whitaker_et_al_AnalChem_2007_v79_p9045_QC.pdf	14696438 07f63987233e68d74df6f9c62ecbad97129269af	no	9
Warnings:					
Information:					
55	Non Patent Literature	White_et_al_BiolCybern_1998_v78_p245_QC.pdf	10893025 97c084758c6492b43210947cf82bdf1400b0056e	no	7
Warnings:					
Information:					

56	Non Patent Literature	White_et_al_AnalChem_1996_v68_p2191_QC.pdf	16924442 441157bc33172f62264d5e5f427c35b336c5902b	no	12
Warnings:					
Information:					
57	Non Patent Literature	Wilson_et_al_AACR_Meeting_Poster_2011_1pg_QC.pdf	3577101 f77a6af794a6ece396380bd988cdb3c42ac4fd9c	no	1
Warnings:					
Information:					
58	Non Patent Literature	Wilson_et_al_Clin_Chem_2011_v57_p1712_QC.pdf	2936418 ad9a675e000463cba0dad6c770c869d246f79ddd	no	18
Warnings:					
Information:					
59	Non Patent Literature	Wilson_Abst_Poster_AAN_Annual_Mtg_2011_2pgs_QC.pdf	4141588 f1c1e5689bb1ab3eee615681d5ec76cfc74a294e	no	2
Warnings:					
Information:					
60	Non Patent Literature	Wilson_et_al_Abstract_Poster_AACC_2014_2pgs.pdf	849236 4489a121c0f870b15982a5bb9c15d2c7a336f9a9	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	514439887		

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

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/111,326	06/24/2014	David Wilson	Q0052.70018US01	3274

23628 7590 05/14/2015
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

EXAMINER
WEIDNER, ADAM M

ART UNIT	PAPER NUMBER
1649	

NOTIFICATION DATE	DELIVERY MODE
05/14/2015	ELECTRONIC

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

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

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

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5219

Notice of Non-Compliant Amendment (37 CFR 1.121)	Application No. 14/111,326	Applicant(s) WILSON ET AL.
	Examiner ADAM M. WEIDNER	Art Unit 1649

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

The amendment document filed on *28 April 2015* is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121 or 1.4. In order for the amendment document to be compliant, correction of the following item(s) is required.

THE FOLLOWING MARKED (X) ITEM(S) CAUSE THE AMENDMENT DOCUMENT TO BE NON-COMPLIANT:

- ☐ 1. Amendments to the specification:
  - ☐ A. Amended paragraph(s) do not include markings.
  - ☐ B. New paragraph(s) should not be underlined.
  - ☐ C. Other _____.
- ☐ 2. Abstract:
  - ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
  - ☐ B. Other _____.
- ☐ 3. Amendments to the drawings:
  - ☐ A. The drawings are not properly identified in the top margin as "Replacement Sheet," "New Sheet," or "Annotated Sheet" as required by 37 CFR 1.121(d).
  - ☐ B. The practice of submitting proposed drawing correction has been eliminated. Replacement drawings showing amended figures, without markings, in compliance with 37 CFR 1.84 are required.
  - ☐ C. Other _____.
- ☒ 4. Amendments to the claims:
  - ☐ A. A complete listing of all of the claims is not present.
  - ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
  - ☐ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following status identifiers: (Original), (Currently amended), (Canceled), (Previously presented), (New), (Not entered), (Withdrawn) and (Withdrawn-currently amended).
  - ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
  - ☒ E. Other: *See Continuation Sheet*.
- ☐ 5. Other (e.g., the amendment is unsigned or not signed in accordance with 37 CFR 1.4):

_____

For further explanation of the amendment format required by 37 CFR 1.121, see MPEP § 714.

TIME PERIODS FOR FILING A REPLY TO THIS NOTICE:

1. Applicant is given **no new time period** if the non-compliant amendment is an after-final amendment or an amendment filed after allowance. If applicant wishes to resubmit the non-compliant after-final amendment with corrections, the **entire corrected amendment** must be resubmitted.
2. Applicant is given **two months** from the mail date of this notice to supply the correction, if the non-compliant amendment is one of the following: a preliminary amendment, a non-final amendment (including a submission for a request for continued examination (RCE) under 37 CFR 1.114), a supplemental amendment filed within a suspension period under 37 CFR 1.103(a) or (c), and an amendment filed in response to a *Quayle* action. If any of above boxes 1. to 4. are checked, the correction required is only the **corrected section** of the non-compliant amendment in compliance with 37 CFR 1.121.

   Extensions of time are available under 37 CFR 1.136(a) only if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action.

   Failure to timely respond to this notice will result in:
   Abandonment of the application if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action; or
   Non-entry of the amendment if the non-compliant amendment is a preliminary amendment or supplemental amendment.

/ADAM M WEIDNER/ Examiner, Art Unit 1649	

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5220

Continuation of 4(e) Other: Applicant indicates new claims 92-112 have been added. However, the claims submitted go from claim 103 to claim 106. Claim 106 references claim 105, which does not exist nor has it ever existed. Claims 104 and 105 are unaccounted for and it is unclear if Applicant meant to include two additional claims or if the claim numbers are simply erroneous.

Notice of Non-Compliant Amendment (37 CFR 1.121)	Application No. 14/111,326	Applicant(s) WILSON ET AL.
	Examiner ADAM M. WEIDNER	Art Unit 1649

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

The amendment document filed on *28 April 2015* is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121 or 1.4. In order for the amendment document to be compliant, correction of the following item(s) is required.

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- ☐ 1. Amendments to the specification:
  - ☐ A. Amended paragraph(s) do not include markings.
  - ☐ B. New paragraph(s) should not be underlined.
  - ☐ C. Other _____.
- ☐ 2. Abstract:
  - ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
  - ☐ B. Other _____.
- ☐ 3. Amendments to the drawings:
  - ☐ A. The drawings are not properly identified in the top margin as "Replacement Sheet," "New Sheet," or "Annotated Sheet" as required by 37 CFR 1.121(d).
  - ☐ B. The practice of submitting proposed drawing correction has been eliminated. Replacement drawings showing amended figures, without markings, in compliance with 37 CFR 1.84 are required.
  - ☐ C. Other _____.
- ☒ 4. Amendments to the claims:
  - ☐ A. A complete listing of all of the claims is not present.
  - ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
  - ☐ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following status identifiers: (Original), (Currently amended), (Canceled), (Previously presented), (New), (Not entered), (Withdrawn) and (Withdrawn-currently amended).
  - ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
  - ☒ E. Other: *See Continuation Sheet*.
- ☐ 5. Other (e.g., the amendment is unsigned or not signed in accordance with 37 CFR 1.4):

_____

For further explanation of the amendment format required by 37 CFR 1.121, see MPEP § 714.

TIME PERIODS FOR FILING A REPLY TO THIS NOTICE:

1. Applicant is given **no new time period** if the non-compliant amendment is an after-final amendment or an amendment filed after allowance. If applicant wishes to resubmit the non-compliant after-final amendment with corrections, the **entire corrected amendment** must be resubmitted.
2. Applicant is given **two months** from the mail date of this notice to supply the correction, if the non-compliant amendment is one of the following: a preliminary amendment, a non-final amendment (including a submission for a request for continued examination (RCE) under 37 CFR 1.114), a supplemental amendment filed within a suspension period under 37 CFR 1.103(a) or (c), and an amendment filed in response to a *Quayle* action. If any of above boxes 1. to 4. are checked, the correction required is only the **corrected section** of the non-compliant amendment in compliance with 37 CFR 1.121.

   Extensions of time are available under 37 CFR 1.136(a) only if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action.

   Failure to timely respond to this notice will result in:
   Abandonment of the application if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action; or
   Non-entry of the amendment if the non-compliant amendment is a preliminary amendment or supplemental amendment.

/ADAM M WEIDNER/ Examiner, Art Unit 1649	

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5222

Continuation of 4(e) Other: Applicant indicates new claims 92-112 have been added. However, the claims submitted go from claim 103 to claim 106. Claim 106 references claim 105, which does not exist nor has it ever existed. Claims 104 and 105 are unaccounted for and it is unclear if Applicant meant to include two additional claims or if the claim numbers are simply erroneous.

Docket No.: Q0052.70018US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	David Wilson
Application No.:	14/111,326
Confirmation No.:	3274
Filed:	June 24, 2014
For:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
Examiner:	A. M. Weidner
Art Unit:	1649

Certificate of Electronic Filing Under 37 CFR 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 3, 2015 Electronic Signature for: /Eileen M. MacKenzie/

RESPONSE TO NOTICE OF NON-COMPLIANT AMENDMENT

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

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Notice of Non-Compliant Amendment Office Action dated May 14, 2015, please replace the AMENDMENTS TO THE CLAIMS section beginning on page 3 of the AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. 1.111 electronically filed on April 28 with the corrected section presented below indicating the corrections with respect to the defective claim listing submitted with the prior submission indicated according to the requirements of 37 C.F.R. § 1.121.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1-54. (Cancelled)

55. (Original) A method of determining a method of treatment for and/or a prognosis of a patient's recovery from a brain injury, comprising:

(a) performing an assay on each of a plurality of samples obtained from the patient following the brain injury to determine the measured concentration of tau protein in each of the samples, wherein the plurality of samples are obtained from the patient over a period of time of at least about 48 hours;

(b) determining the area under the curve of a graph of the tau protein concentration in the plurality of samples versus time, wherein the area is determined for the entire time period and/or for a second peak in the tau protein concentration; and

(c) determining a prognosis of the patient's recovery from the brain injury and/or a method of treatment based at least in part on the area under the curve for the entire time period and/or the second peak in the tau protein concentration determined in step (b).

56-91. (Cancelled)

92. (Previously presented) The method of claim 55, wherein the brain injury results from a hypoxic event.

93. (Previously presented) The method of claim 55, wherein the limit of detection for the assay is less than about 0.2 pg/mL.

94. (Previously presented) The method of claim 55, wherein when the area under the curve for the entire period of time is less than about 800, the prognosis is good based on the CPC scale.

95. (Previously presented) The method of claim 55, wherein when the area under the curve for the entire period of time is greater than about 800, the prognosis is poor based on the CPC scale.

96. (Previously presented) The method of claim 55, wherein when the area under the curve for the second peak in the tau protein concentration is less than about 500, the prognosis is good based on the CPC scale.

97. (Previously presented) The method of claim 55, wherein when the area under the curve for the second peak in the tau protein concentration is greater than about 500, the prognosis is poor based on the CPC scale.

98. (Previously presented) The method of claim 55, wherein the baseline concentration for determining the area under the curve for the entire time period is equal to zero.

99. (Previously presented) The method of claim 55, wherein the baseline concentration for determining the area under the curve of the second peak of tau protein concentration is equal to the concentration of tau protein present in the sample obtained from the patient substantially immediately prior to a second increase in tau protein concentration.

100. (Previously presented) The method of claim 55, wherein tau protein is measured in bodily fluid.

101. (Previously presented) The method of claim 100, wherein the bodily fluid is blood.

102. (Previously presented) The method of claim 100, wherein the bodily fluid is not CSF.

103. (Previously presented) The method of claim 55, wherein the assay comprises:

spatially segregating at least a portion of the tau protein molecules into a plurality of separate locations;

addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of said locations containing a tau protein molecule; and

determining a measure of the concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain a tau protein molecule.

[[106]]104. (Currently amended) The method of claim [[105]]103, further comprising exposing the plurality of tau protein molecules to a plurality of binding ligands such that at least some of the tau protein molecules associate with a single binding ligand and a statistically significant fraction of the tau protein molecules do not associate with any binding ligand.

[[107]]105. (Currently amended) The method of claim 55, wherein the assay comprises:

immobilizing tau protein molecules with respect to a plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

spatially segregating at least a portion of the capture objects subjected to the immobilizing step into a plurality of separate locations;

addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of said locations containing a tau protein molecule; and

determining a measure of the concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain a tau protein molecule.

[[108]]106. (Currently amended) The method of claim 55, wherein the assay comprises:

exposing a plurality of capture objects that each include a binding surface having affinity for at least one tau protein molecule, to a solution containing or suspected of containing the at least one type of tau protein molecule;

immobilizing tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

spatially segregating at least a portion of the capture objects subjected to the immobilizing step into a plurality of separate locations;

addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of said locations containing a tau protein molecule; and

determining a measure of the concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain a tau protein molecule.

[[109]]107. (Currently amended) A method of claim 55, wherein the assay comprises:

exposing a plurality of capture objects that each include a binding surface having affinity for at least one type of tau protein molecule, to a solution containing or suspected of containing the at least one type of tau protein molecule to form capture objects comprising at least one immobilized tau protein molecule;

mixing the capture objects prepared in the exposing step to a plurality of binding ligands such that at least some of the capture objects associate with a single binding ligand and a statistically significant fraction of the capture objects do not associate with any binding ligand;

spatially segregating at least a portion of the capture objects subjected to the mixing step into a plurality of locations;

addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of locations containing a binding ligand; and

determining a measure of the concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain a binding ligand.

[[110]]108. (Currently amended) The method of claim 103, wherein the plurality of locations comprises a plurality of reaction vessels.

[[111]]109. (Currently amended) The method of claim [[110]]108, wherein the average volume of the plurality of reaction vessels is between about 10 attoliters and about 100 picoliters.

[[112]]110. (Currently amended) The method of claim 103, wherein the measure of the concentration of tau protein molecules in the fluid sample is determined at least in part by comparison of a measured parameter to a calibration standard.

REMARKS

Applicant responds to the Notice of Non-Compliant Amendment mailed by the Patent Office on May 14, 2015.

Claims 106-112 have been amended to correct erroneous claim numbering. No new matter has been added.

No fee is believed due with this response. However, if a fee is due, please charge our Deposit Account No. 23/2825 under Docket No. Q0052.70018US01 from which the undersigned is authorized to draw.

Dated: June 3, 2015

Respectfully submitted,

By: /Michael J. Pomianek /
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
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Boston, Massachusetts 02210-2206
617.646.8000

Doc Code: TRAN.LET
Document Description: Transmittal Letter

USED IN LIEU OF PTO/SB/21 (07-09)

TRANSMITTAL FORM (to be used for all correspondence after initial filing)			
		Application Number	14/111,326-Conf. #3274
		Filing Date	June 24, 2014
		First Named Inventor	David Wilson
		Art Unit	1649
		Examiner Name	A. M. Weidner
Total Number of Pages in This Submission		Attorney Docket Number	Q0052.70018US01

ENCLOSURES (*Check all that apply*)

☐ Fee Transmittal Form	☐ Drawing(s)	☐ After Allowance Communication to TC
☐ Fee Attached	☐ Licensing-related Papers	☐ Appeal Communication to Board of Appeals and Interferences
☐ Amendment/Reply	☐ Petition	☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
☐ After Final	☐ Petition to Convert to a Provisional Application	☐ Proprietary Information
☐ Affidavits/declaration(s)	☐ Power of Attorney, Revocation Change of Correspondence Address	☐ Status Letter
☐ Extension of Time Request	☐ Terminal Disclaimer	☒ Other Enclosure(s) (please Identify below):
☐ Express Abandonment Request	☐ Request for Refund	Response to Non-Compliant Amendment
☐ Information Disclosure Statement	☐ CD, Number of CD(s) _____	
☐ Certified Copy of Priority Document(s)	☐ Landscape Table on CD	
☐ Reply to Missing Parts/ Incomplete Application	Remarks	
☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	**June 3, 2015**	Reg. No.	46,190

Certificate of Electronic Filing Under 37 CFR 1.8

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Dated: June 3, 2015 Electronic Signature for: /Eileen M. MacKenzie/

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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/111,326	Filing Date 06/24/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	06/03/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/VERONICA DAY EVERETT/

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

EFS ID:	22530999
Application Number:	14111326
International Application Number:	
Confirmation Number:	3274
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Eileen MacKenzie
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018US01
Receipt Date:	03-JUN-2015
Filing Date:	24-JUN-2014
Time Stamp:	20:41:04
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Q005270018US01-TRN-MJP.pdf	24773 a1759eada40b1eb05357a2e6fbf9572236a8a0ca	no	1

Warnings:	
Information:	

2		Q005270018US01-RNNC-MJP.pdf	40986 943121a171211cf71d1a7aaa8a8191e8a1bd673c	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	2
	Claims	3	7
	Applicant Arguments/Remarks Made in an Amendment	8	8

Warnings:	
Information:	
Total Files Size (in bytes):	65759

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/111,326	06/24/2014	David Wilson	Q0052.70018US01	3274

23628 7590 09/21/2015

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BOSTON, MA 02210-2206

EXAMINER
WEIDNER, ADAM M

ART UNIT	PAPER NUMBER
1649	

NOTIFICATION DATE	DELIVERY MODE
09/21/2015	ELECTRONIC

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

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

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

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5236

Office Action Summary	Application No. 14/111,326	Applicant(s) WILSON ET AL.	
	Examiner ADAM M. WEIDNER	Art Unit 1649	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on 6/3/15.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

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

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) 55 and 92-110 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) 55 and 92-110 is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5237

The present application is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

DETAILED ACTION

This action is in response to claim amendments filed 6/3/15 as well as arguments filed 4/28/15. Claims 55 and 92-110 are pending and under examination, with claims 92-110 being newly presented (see notice of non-compliance mailed 5/14/15).

Withdrawn Rejections

The objection to the specification is withdrawn.

The claim objections are moot as the claims have been cancelled.

The rejections under §112 are moot as the claims have been cancelled.

The rejections under §103 are withdrawn. Applicant argues that the only independent claim (claim 55) requires determining the area under the curve of a graph of the tau protein concentration. Applicant argues that there is nothing in the cited art to suggest using this particular method of analysis over any other method of analysis, and that this protocol/algorithm is used to determine a prognostic rating. Applicant argues that "these correlations or their ability to be successfully exploited in a method of treatment for and/or a prognosis of a patient's recovery from a brain injury" would not have been predictable or expected (Remarks 4/28/15; p.11). This is persuasive. The double patenting rejection is withdrawn for the same reason.

New Rejections Necessitated by Amendment

Claim Objections

Claims 94-97 are objected to because of the following informalities: values should be accompanied by the appropriate units. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 99 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The term "substantially" is a relative term which renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The claim requires obtaining a sample “substantially immediately” prior to a second increase in tau protein concentration. The term “substantially" is used throughout the specification in different contexts with no particular definition. On p.13 an example is provided (prior to the concentration of the biomarker increasing due to the hypoxic event) but this may be an example of either “immediately" or “substantially immediately”; the specification does not make this clear. Thus, the metes and bounds of the term cannot be determined (i.e., what is immediate versus substantially immediate versus not substantially immediate?).

Therefore, claim 99 is indefinite.

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 93 is rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention. The claim is drawn to the genus of all assays which meet a certain detection threshold, such as 0.2 pg/ml. However, the specification only discloses a single assay which meets this limitation (digital ELISA) and there are no references of record which indicate any other assay which meets this limitation. Thus, by only providing a single example and no guidance with respect to other assays with this detection limit, Applicant has not readily conveyed to the skilled artisan that Applicant was in possession of a sufficient number of such assays to be entitled to claiming the entire genus of all assays with such a detection limit. It is noted than many assays which "employ spatially segregating analyte molecules" are listed in the specification (p.22-23); however, the specification does not indicate that these assays are capable of achieving a detection limit of 0.2 pg/ml. Even *arguendo* if they were, these represent at best the methods known to Applicant, and does not provide sufficient support to claim any and all assays which meet this detection limit.

Response to Arguments

Applicant's arguments filed 4/28/15 have been fully considered but they are not persuasive. This rejection was made over previous claims that recited the same limitation, and Applicant's sole argument is that these claims have been cancelled. While this renders the rejection of the cancelled claims moot, it does not address the rejection itself.

Therefore, claim 93 does not meet the written description requirement.

Claims 104-107 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims require using "binding ligands", "capture objects", and "binding surface". These terms all denote something that has an affinity for tau and in some cases binds certain tau but not others. In making a determination of whether the application complies with the written description requirement of 35 U.S.C. § 112(a), or 35 U.S.C. §112 first paragraph (Pre-AIA), it is necessary to understand what Applicant has possession of and what Applicant is claiming. However, the examples in the specification are not sufficient to convey to the artisan that Applicant was in possession of a sufficient number of species to warrant a claim to this broad genus.

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof. In this case, the terms are defined solely by a function; there is no identification of any particular structure that must be conserved throughout the genus. “Binding ligand” is defined on p.27 of the specification as “any molecule, particle, or the like which specifically binds to or otherwise specifically associates with a biomarker molecule to aid in the detection of the biomarker molecule". Thus the ligand has no structure associated with it and even its function is defined vaguely, "specifically binding” or “specifically associating” with any possible biomarker such that it “aids in the detection” of the biomarker. The claim itself would limit the biomarker to tau, as it is tau being detected in the claimed method. However, the claim requires that not only does the ligand "associate" with "at least some of the tau protein molecules", it also requires that it does not associate with a statistically significant fraction of the tau protein molecules. There is no indication of any particular structure responsible for this function and as such, the skilled artisan cannot immediately envision the detailed chemical structure responsible

nor enough members of this genus to convey to the skilled artisan that Applicant was in possession of this genus. Accordingly, in the absence of sufficient recitation of distinguishing identifying characteristics, the specification does not provide adequate written description of the claimed genus.

No specific definition of "capture object" is provided and the examples in the specification (e.g., bead) do not convey possession of the entire genus of such objects for the reasons above.

No definition of "binding surface" is provided. This term appears to define a functional portion of a capture object (p.23) but does not provide any further structural characteristics.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states "applicant must convey with reasonable clarity to those skilled in the art that, as of filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See Vas-Cath at page 1116). As discussed above, the artisan cannot envision the detailed chemical structure of the objects encompassed by the terms, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The compound itself is required. See Fiers v. Revel, 25 USPQ2d 1601 at 1606 (CAFC 1993) and Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016.

One cannot describe what one has not conceived. See Fiddes v. Baird, 30 USPQ2d 1481 at 1483. In Fiddes, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Applicant is reminded that Vas Cath makes clear that the written description provision of 35 U.S.C. § 112 is severable from its enablement provision (see page 1115).

Therefore, claims 104-107 do not meet the written description requirement.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 55 and 92-110 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more. The claims are directed a series of steps (a method) for measuring a natural concentration of naturally occurring tau protein. The claims do not include additional elements that are sufficient to amount to significantly more than observing a natural phenomenon. Note that this rejection is considered maintained over claim 55 and necessitated by amendment for newly added claims 92-110.

Claim 55 is an independent claim. It is generally drawn to performing any assay on any samples following any brain injury to measure tau, so long as it is done more than once within 48 hours of the injury, followed by data analysis (Area Under the Curve), and culminating in generic instructions that this information should be used to determine a prognosis or inform a treatment decision in some way. This is no more than observing the natural phenomenon with general instructions to "apply it". This is similar to the claims at issue in Mayo, in which the Supreme Court indicated that a correlation that is a consequence of how a certain compound is metabolized was patent ineligible. Observation that the natural expression of tau is somehow correlated to brain injury with general instructions that the information should be somehow "applied" does not add significantly more to the exception itself and is thus patent ineligible. The instant claim includes mentally determining a treatment protocol or prognosis of a patient's recovery from brain injury by measuring tau in any patient sample using any means and using this information to somehow inform such a decision. There is no particular machine or any limitations that add significantly more than a simple observation of a naturally occurring protein at its natural levels with generic instructions to "apply it".

With respect to claim 92, limiting the injury to one which results from a hypoxic event is simply further defining the population from which to gather data, i.e., further describing where the correlation is found. This does not add significantly more to the correlation itself.

With respect to claim 93, introduction of an assay detection limit does not require any specific machine or transformation, nor does it even relate to the correlation itself as the claim does not require any particular value of tau is measured, only that the assay should be capable of detecting tau at a certain threshold. This does not add significantly more to the correlation.

With respect to claims 94-97, these claims describe certain threshold values where a prognosis is "good" or "poor". However, these claims are descriptions of the data itself. This is similar to the fact pattern in Mayo. In Mayo, a level of the metabolite "less than about 230 pmol per $8x10^8$ red blood cells [indicated] a need to increase the amount of said drug". Such claim limitations were found to be a description of the correlation itself and not "significantly more". It was also found that steps prior to this constituted routine data gathering, instructing the artisan to engage in conventional, well-known practices and then "warning" the user of the implications of the data; this was not eligible in Mayo and thus the instant claim(s) are not patent eligible.

With respect to claims 98-99, these claims describe the baseline concentration. As this only serves to further define data gathering/the correlation, these claims do not offer significantly more.

With respect to claims 100-102, these claims indicate the sample should be a body fluid, particularly blood or "not CSF" (e.g., blood). However, these are well-known fluids in which to measure tau protein and represent a routine, conventional practice. These steps represent "mere data gathering" in conjunction with a law of nature or abstract idea, and is considered "insignificant extrasolution activity" (see guidelines in Federal Register indicated above).

With respect to claims 103-110, these claims are also directed to the data gathering step. Limitations regarding how tau is to be measured represents insignificant data-gathering steps, insignificant pre/extra-solution activity, and an attempt to limit the use of the abstract idea/correlation to a particular technological environment (See Federal Register p.74632). Viewed both individually and as an ordered combination, the claimed steps are conventional and specified at a high level of generality. There is no suggestion that these data gathering steps are themselves novel and the instant specification points to numerous examples of existing technology for this purpose (e.g., p.22-23, 25, 34).

In summary, the above analysis conducted in line with the guidance issued on 12/15/2014 sets forth the rationale for deeming the above claims patent ineligible. It is appreciated that all inventions at some level embody, use, reflect, rest upon, or apply a law of nature, natural phenomenon, or abstract idea. However, the instant claims do not improve another technology, apply the judicial exception with any specific machine, transform the gathered data into a different state or thing, add a limitation that is anything more than what is well-understood, conventional, or routine in the field, or any other meaningful limitation beyond generally linking the use of the exception to a particular technological environment. In other words, the claims as a whole instruct a user to gather tau using any number of known methods and using conventional samples and then informs this relevant audience about a correlation that exists between the gathered data and a prognosis. The added steps of determining the prognosis "based at least in part" on the gathered data is no more than instructions to "apply it".

Response to Arguments

Applicant's arguments filed 4/28/15 have been fully considered but they are not persuasive. Applicant argues that claim 55 cannot be fairly viewed as an attempt to "tie up" the judicial exception. Applicant argues that rather the claims relate to one particular application of the judicial exception in one specific way (determining a prognosis) and therefore the claim is eligible.

This has been fully considered but is not persuasive. The natural correlation, even if complex, remains a judicial exception. Thus, claims describing methods of analysis (area under the curve, peaks to measure, baselines to establish, thresholds which indicate information) are all part of the correlation itself. Thus, the limitations outside the judicial exception must provide "significantly more". However, the instant limitations beyond the correlation itself are largely drawn to the data gathering step. This is discussed in the guidelines cited by Applicant, and generally does not add significantly more to the natural correlation itself. Rather, the guidelines look to the application of that law/correlation ("new and useful end", a robotic arm that is controlled by a mathematical relationship, *Bilski*, a machine that uses the Arrhenius equation to improve an existing process such that the gathered information instructs a machine to open at the appropriate time and results in the transformation of one product into another). In the instant case, the application of the correlation is no more than instructions to make a decision based at least in part on the

gathered information. Such a broad, generic limitation is akin to the words "apply it" which does not offer significantly more in the same way that gathering metabolite information and informing the user of the possible need to alter treatment was not eligible.

Therefore, claims 55 and 92-110 are not patent eligible.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ADAM M. WEIDNER whose telephone number is (571)272-3045. The examiner can normally be reached on Monday - Friday, 800 - 1630 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/A. M. W./

Examiner, Art Unit 1649

/GREGORY S EMCH/

Primary Examiner, Art Unit 1649

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14111326	WILSON ET AL.
	Examiner	Art Unit
	ADAM M WEIDNER	1649

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
inventor east	1/22/15	amw
text east/google	1/23/15	amw
PLUS search	1/23/15	amw
updated inventor/assignee east	9/14/15	amw
update text search (AOC, tau, brain injury, diagnosis/prognosis) east, internet	9/14/15	amw

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L11	128	10 and @pd<"20110412"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:10
L10	331	6 and (diagnos$ or prognos$) and ((brain injury) or hypoxia or hypoxic or TBI or concussion)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:10
L9	64	8 and @pd<"20110412"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:06
L8	128	7 and tau.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:06
L7	1692	6 and (diagnos$ or prognos$)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:05
L6	2771	5 and tau	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:05
L5	48615	(area under) with curve	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:04
L4	19	3 and (area under)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:01
L3	64	(1 or 2) and tau	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:01
L2	6692	((wilson near2 david) or (john near2 zetterber) or (kaj near2 blennow) or (jeffrey near2 randall)).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:01
L1	20	banyan biomarkers.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2015/09/14 11:00

9/ 14/ 2015 11:12:25 AM
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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5249

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5250

Today - Monday, September 14, 2015

10:59 AM Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initia... www.ncbi.nlm.nih.gov

10:59 AM https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&cad=rja&u... www.google.com

10:58 AM Mild therapeutic hypothermia alters neuron specific enolase as an outcome predic... www.ncbi.nlm.nih.gov

10:58 AM https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&u... www.google.com

10:58 AM "area under the curve" tau protein CPC - Google Search www.google.com

10:58 AM JAMA Network | JAMA Neurology | Cerebrospinal Fluid Tau and β-Amyloid ... archneur.jamanetwork.com

10:58 AM Negative correlation between cerebrospinal fluid tau protein and cognitive functi... onlinelibrary.wiley.com

10:58 AM https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=6&cad=rja&u... www.google.com

10:58 AM https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&cad=rja&u... www.google.com

10:58 AM "area under the curve" tau protein - Google Search www.google.com

10:57 AM "area under the curve" tau protein - Google Search www.google.com

10:57 AM "area under the curve" tau - Google Search www.google.com

10:00 AM PEER Extended efi.uspto.gov

9:17 AM Mayo Collaborative Servs. v. Prometheus Labs., Inc. - Wikipedia, the free encyclopedia en.wikipedia.org

9:17 AM mayo claims indicate 101 - Google Search www.google.com

9:17 AM mayo claims indicate - Google Search www.google.com

9:12 AM area under the curve units - Google Search www.google.com

PTO/SB/30 (07-14)
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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	14/111,326-Conf. #3274
Filing Date	June 24, 2014
First Named Inventor	David Wilson
Art Unit	1649
Examiner Name	A. M. Weidner
Attorney Docket Number	Q0052.70018US01

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.

Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, to any international application that does not compy with the requirements of 35 U.S.C 371, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO on page 2.)

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).
   - a. [ ] Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
     - i. [ ] Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - ii. [ ] Other _____
   - b. [X] Enclosed
     - i. [X] Amendment/Reply
     - ii. [ ] Affidavit(s)/ Declaration(s)
     - iii. [ ] Information Disclosure Statement (IDS)
     - iv. [X] Other Petition for 2-month Extension of Time
2. Miscellaneous
   - a. [ ] Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. [ ] Other _____
3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. [X] The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 23/2825 .
     - i. [X] RCE fee required under 37 CFR 1.17(e)
     - ii. [X] Extension of time fee (37 CFR 1.136 and 1.17)
     - iii. [ ] Other _____
   - b. [ ] Check in the amount of $ _____ enclosed
   - c. [X] Payment by credit card (~~Form PTO-2038 enclosed~~)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Andrea J. Gabert/	Date	February 22, 2016
Name (Print/Type)	Andrea J. Gabert, Ph.D.	Registration No.	68,904

Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 22, 2016 Signature: /Ellen J. Paul/ (Ellen J. Paul)

4604552.1

Docket No.: Q0052.70018US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	David Wilson
Application No.:	14/111,326
Confirmation No.:	3274
Filed:	June 24, 2014
For:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
Examiner:	A. M. Weidner
Art Unit:	1649

Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 22, 2016 Signature: /Ellen J. Paul/ (Ellen J. Paul)

AMENDMENT AFTER FINAL ACTION UNDER 37 C.F.R. § 1.116

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

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated September 21, 2015, finally rejecting claims 55 and 92-110, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5253

AMENDMENTS TO THE CLAIMS

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1-54. (Cancelled)

55. (Currently amended) A method of determining a method of treatment for and/or a prognosis of a patient's recovery from a brain injury, comprising:

(a) performing an assay on each of a plurality of samples obtained from the patient following the brain injury to determine the measured concentration of tau protein in each of the samples, wherein the plurality of samples are obtained from the patient over a period of time of at least about 48 hours;

(b) determining the area under the curve of a graph of the tau protein concentration in the plurality of samples versus time, wherein the area is determined for the entire time period and/or for a second peak in the tau protein concentration; and

(c) determining a prognosis of the patient's recovery from the brain injury and/or a method of treatment based at least in part on the area under the curve for the entire time period and/or the second peak in the tau protein concentration determined in step (b); wherein the assay comprises the steps:

(1) exposing a plurality of beads that are monomeric and covalently associated with anti-tau antibodies to the sample to form beads comprising at least one immobilized tau protein molecule;

(2) mixing the beads prepared in the exposing step with a plurality of monoclonal anti-tau antibody and biotin conjugates, such that at least some of the beads associate with an anti-tau antibody and biotin conjugate and a statistically significant fraction of the beads do not associate with any anti-tau antibody and biotin conjugates;

(3) spatially segregating at least a portion of beads subjected to the mixing step into a plurality of locations;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5254

(4) addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of locations containing an anti-tau antibody and biotin conjugate, wherein the locations containing an anti-tau antibody and biotin conjugate are determined, in part, by exposure of the anti-tau antibody and biotin conjugate to a streptavidin-enzyme conjugate; and

(5) determining the measured concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain an anti-tau antibody and biotin conjugate.

56-91. (Cancelled)

92. (Previously presented) The method of claim 55, wherein the brain injury results from a hypoxic event.

93. (Previously presented) The method of claim 55, wherein the limit of detection for the assay is less than about 0.2 pg/mL.

94. (Currently amended) The method of claim 55, wherein when the area under the curve for the entire period of time is less than about 800 pg.hr/mg, the prognosis is good based on the CPC scale.

95. (Currently amended) The method of claim 55, wherein when the area under the curve for the entire period of time is greater than about 800 pg.hr/mg, the prognosis is poor based on the CPC scale.

96. (Currently amended) The method of claim 55, wherein when the area under the curve for the second peak in the tau protein concentration is less than about 500 pg.hr/mg, the prognosis is good based on the CPC scale.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5255

97. (Currently amended) The method of claim 55, wherein when the area under the curve for the second peak in the tau protein concentration is greater than about 500 pg.hr/mg, the prognosis is poor based on the CPC scale.

98. (Previously presented) The method of claim 55, wherein the baseline concentration for determining the area under the curve for the entire time period is equal to zero.

99. (Currently amended) The method of claim 55, wherein the baseline concentration for determining the area under the curve of the second peak of tau protein concentration is equal to the concentration of tau protein present in the sample obtained from the patient ~~substantially immediately~~ prior to a second increase in tau protein concentration.

100. (Previously presented) The method of claim 55, wherein tau protein is measured in bodily fluid.

101. (Previously presented) The method of claim 100, wherein the bodily fluid is blood.

102. (Previously presented) The method of claim 100, wherein the bodily fluid is not CSF.

103-107. (Cancelled)

108. (Currently Amended) The method of claim 55[[103]], wherein the plurality of locations comprises a plurality of reaction vessels.

109. (Previously presented) The method of claim 108, wherein the average volume of the plurality of reaction vessels is between about 10 attoliters and about 100 picoliters.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5256

110. (Currently amended) The method of claim 55[[103]], wherein the measure of the concentration of tau protein molecules in the fluid sample is determined at least in part by comparison of a measured parameter to a calibration standard.

111. (New) A method for determining a measure of the concentration of tau protein in a blood plasma or serum sample obtained from a patient containing or suspected of containing tau protein, comprising:

performing an assay to determine a measure of the concentration of tau protein in the blood plasma or serum sample, wherein the assay comprises the steps:

(1) exposing a plurality of beads that are monomeric and covalently associated with anti-tau antibodies to the blood plasma or serum sample to form beads comprising at least one immobilized tau protein molecule;

(2) mixing the beads prepared in the exposing step with a plurality of monoclonal anti-tau antibody and biotin conjugates, such that at least some of the beads associate with an anti-tau antibody and biotin conjugate and a statistically significant fraction of the beads do not associate with any anti-tau antibody and biotin conjugates;

(3) spatially segregating at least a portion of beads subjected to the mixing step into a plurality of locations;

(4) addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of locations containing an anti-tau antibody and biotin conjugate, wherein the locations containing an anti-tau antibody and biotin conjugate are determined, in part, by exposure of the anti-tau antibody and biotin conjugate to a streptavidin-enzyme conjugate; and

(5) determining the measured concentration of tau protein molecules in the blood plasma or serum sample based at least in part on the number of locations determined to contain an anti-tau antibody and biotin conjugate.

112. (New) The method of claim 111, wherein the plurality of locations comprises a plurality of reaction vessels.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5257

113. (New) The method of claim 112, wherein the average volume of the plurality of reaction vessels is between about 10 attoliters and about 100 picoliters.

114. (New) The method of claim 111, wherein the measure of the concentration of tau protein molecules in the fluid sample is determined at least in part by comparison of a measured parameter to a calibration standard.

115. (New) The method of claim 111, wherein the limit of detection for the assay is less than about 0.2 pg/mL.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5258

REMARKS

Reconsideration is respectfully requested in view of the amendments above and the remarks below. Claims 55 and 92-110 were previously pending in this application. Claims 103-107 have been canceled. Claims 55, 94-97, 99, 108, and 110 have been amended. Claims 111-115 have been added. Support for the amendments and the new claims may be found throughout the specification as originally filed, for example, see Example 1, Figure 4, and claim 1, 7, and 71-77. As a result, claims 55, 92-102, and 108-115 are now pending for examination, with claims 55 and 111 being independent claims. No new matter has been added.

Objections to Claims 94-97

Claims 94-97 are objected to because of the following informalities: "values should be accompanied by the appropriate units." Claims 94-97 have been amended to recite pg.hr/mg. Accordingly, withdrawal of the claim objections on this ground is requested.

Rejections Under 35 U.S.C. §112

Claim 99 is rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. Without acceding to the merits of the rejection, the term "substantially" has been deleted from claim 99.

Claim 93 is rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. Without acceding to the merits of the rejection, additional details regarding the assay has been recited in claim 55 from which claim 93 depends to obviate the rejection.

Claims 104-107 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. Without acceding to the merits of the rejection, claims 104-107 have been cancelled. It is noted that the objected to terms are not present in amended claim 55.

Accordingly, withdrawal of the claim rejections on this ground is requested

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5259

Rejections Under 35 U.S.C. §101

Claims 55 and 92-110 are rejected under 35 U.S.C. §101 because the claimed invention is alleged to be directed to a judicial exception (i.e., a law of nature, a natural phenomenon, or an abstract idea) without significantly more.

Without acceding to the merits of the rejection and solely in the interest of advancing prosecution, claim 55 has been amended to recite multiple innovative assay steps and details regarding the assay components that are not conventional and that were specifically developed to facilitate the detection of tau antibodies in the context of the presently claim inventions. As a whole (collectively including each and every step), claim 55 does not merely recite or preempt a law of nature, a natural phenomenon, or an abstract idea without providing significantly more. Claim 55 recites a new and innovative assay comprising steps of (1) exposing a plurality of beads that are monomeric and covalently associated with anti-tau antibodies to the sample to form beads comprising at least one immobilized tau protein molecule; (2) mixing the beads prepared in the exposing step with a plurality of monoclonal anti-tau antibody and biotin conjugates, such that at least some of the beads associate with an anti-tau antibody and biotin conjugate and a statistically significant fraction of the beads do not associate with any anti-tau antibody and biotin conjugates; (3) spatially segregating at least a portion of beads subjected to the mixing step into a plurality of locations; (4) addressing at least a portion of the plurality of locations subjected to the spatially segregating step and determining the number of locations containing an anti-tau antibody and biotin conjugate, wherein the locations containing an anti-tau antibody and biotin conjugate are determined, in part, by exposure of the anti-tau antibody and biotin conjugate to a streptavidin-enzyme conjugate; and (5) determining the measured concentration of tau protein molecules in the sample based at least in part on the number of locations determined to contain an anti-tau antibody and biotin conjugate. Beads that are covalently associated with anti-tau antibodies, monoclonal anti-tau antibody and biotin conjugates, and streptavidin-enzyme conjugate are not naturally occurring components and thus it follows that the act of an assay using these components cannot occur in nature; it is not a law of nature, a natural phenomenon, or an abstract idea. Thus, claim 55, as a whole, is not solely directed to or preempting a judicial exception without reciting significantly more – i.e. in the nature of novel and unconventional assay techniques and components purposefully

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5260

directed to achieving the unprecedented performance and capabilities of the presently claimed methods for detecting tau protein and treatment and/or a prognosis of a patient's recovery from a brain injury. Thus, claim 55 is patent-eligible under 35 U.S.C. §101.

Accordingly, withdrawal of this rejection is respectfully requested.

New Claims

New independent claim 111 has been added. New claim 111 recites similar assay steps as recited in claim 55. Accordingly, for at least the reasons stated above in connection with claim 55, claim 111 and the claims that depend therefrom are patent eligible.

CONCLUSION

In view of the above amendment, the pending application is believed to be in condition for allowance.

No fee is believed due with this response. However, if a fee is due, please charge our Deposit Account No. 23/2825 under Docket No. Q0052.70018US01 from which the undersigned is authorized to draw.

Dated: February 22, 2016

Respectfully submitted,

By /Andrea J. Gabert/

Andrea J. Gabert, Ph.D.
Registration No.: 68,904
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5261

PTO/SB/22 (03-13)
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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) Q0052.70018US01
Application Number 14/111,326-Conf. #3274	Filed June 24, 2014
For METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY	
Art Unit 1649	Examiner A. M. Weidner

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☒ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$ 600.00
☐ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

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

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. ~~Form PTO-2038 is attached~~.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 23/2825 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration number 68,904 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Andrea J. Gabert/	February 22, 2016
Signature	Date
Andrea J. Gabert, Ph.D.	617.646.8000
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 22, 2016 Electronic Signature: /Ellen J. Paul/ (Ellen J. Paul)

4599458.1

DOCKET NO.: Q0052.70018US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	David Wilson
Application No.:	14/111,326
Confirmation No.:	3274
Filed:	October 11, 2013
For:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
Examiner:	A. M. Weidner
Art Unit:	1649

CERTIFICATE OF ELECTRONIC FILING UNDER 37 C.F.R. § 1.8

The undersigned hereby certifies that this paper, along with any paper referred to as being attached or enclosed, is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4), on the 22nd day of February, 2016.

_____/Nathaniel W. Taylor/_____
Nathaniel W. Taylor

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

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action after the filing of a Request for Continued Examination under 37 C.F.R. § 1.114.

No fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5263

presentation of the references should not be construed as an indication of the importance of the references.

The undersigned hereby makes the following additional information of record in the above-identified application.

The undersigned would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application:

Serial No.	Filing Date	Inventor(s)	Docket No.
*14/111,331	12-18-2013	Wilson et al.	Q0052.70017US01

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of this application;

3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5264

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees occasioned by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825 under Docket No. Q0052.70018US01 from which the undersigned is authorized to draw.

Respectfully submitted,

By: /Andrea J. Gabert/

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Docket No.: Q0052.70018US01
Date: February 22, 2016
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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5265

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 14/111,326	ATTY. DOCKET NO.: Q0052.70018US01
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: October 11, 2013	CONFIRMATION NO.: 3274
	FIRST NAMED INVENTOR: David Wilson	
Sheet 1 of 3	GROUP ART UNIT: 1649	EXAMINER: A. M. Weidner

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Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
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FORM PTO-1449/A and B (modified PTO/SB/08)				APPLICATION NO.: 14/111,326	ATTY. DOCKET NO.: Q0052.70018US01
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				FILING DATE: October 11, 2013	CONFIRMATION NO.: 3274
				FIRST NAMED INVENTOR: David Wilson	
Sheet	2	of	3	GROUP ART UNIT: 1649	EXAMINER: A. M. Weidner

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				FIRST NAMED INVENTOR: David Wilson	
Sheet	3	of	3	GROUP ART UNIT: 1649	EXAMINER: A. M. Weidner

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Examiner's Initials #	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

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

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 May 2007 (03.05.2007)

PCT

(10) International Publication Number
WO 2007/050359 A2

(51) International Patent Classification:
C07K 16/00 (2006.01)

(21) International Application Number:
PCT/US2006/040508

(22) International Filing Date: 17 October 2006 (17.10.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/US2005/381125
21 October 2005 (21.10.2005) US

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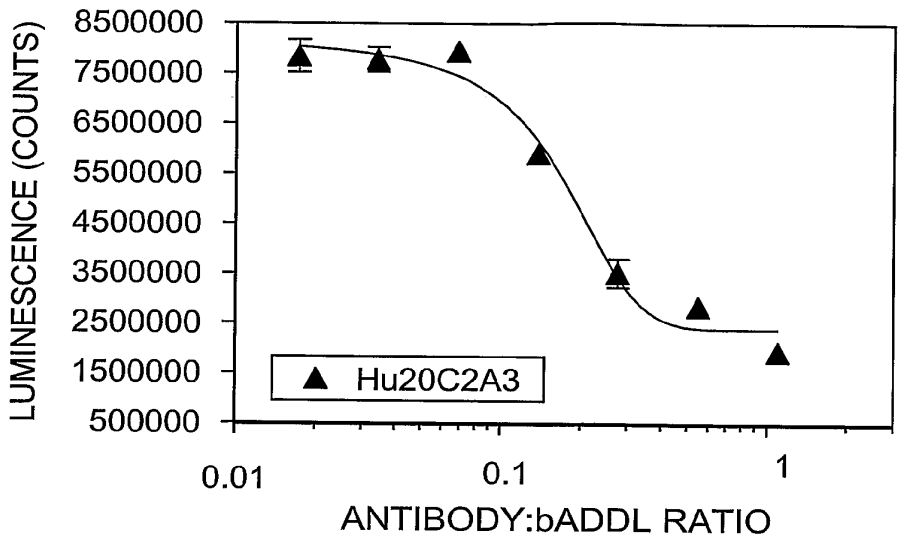
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,

[Continued on next page]

(54) Title: ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF



(57) Abstract: The present invention relates to antibodies that -differentially -recognize- -multi- dimensional conformations- of- Aβ- derived diffusible ligands, also known as ADDLs. The antibodies of the invention can distinguish between Alzheimer's Disease and control human brain extracts and are useful in methods of detecting ADDLs and diagnosing Alzheimer's Disease. The present antibodies also block binding of ADDLs to neurons, assembly of ADDLS, and tau phosphorylation and are there useful in methods for the preventing and treating diseases associated with soluble oligomers of amyloid β 1-42.

WO 2007/050359 A2

RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF

Introduction

This application is a continuation-in-part of PCT Application No. PCT/US2005/0038125 filed October 21, 2005.

Background of the Invention

Alzheimer's Disease is a progressive and degenerative dementia (Terry, et al. (1991) *Ann. Neurol.* 30:572-580; Coyle (1987) In: *Encyclopedia of Neuroscience*, Adelman (ed.), Birkhäuser, Boston-Basel-Stuttgart, pp 29-31,). In its early stages, Alzheimer's Disease manifests primarily as a profound inability to form new memories (Selkoe (2002) *Science* 298:789-791), reportedly due to neurotoxins derived from amyloid beta (Aβ). Aβ is an amphipathic peptide whose abundance is increased by mutations and risk factors linked to Alzheimer's Disease. Fibrils formed from Aβ constitute the core of amyloid plaques, which are hallmarks of an Alzheimer's Disease brain. Analogous fibrils generated *in vitro* are lethal to cultured brain neurons. These findings indicate that memory loss is a consequence of neuron death caused by fibrillar Aβ.

Despite strong experimental support for fibrillar Aβ and memory loss, a poor correlation exists between dementia and amyloid plaque burden (Katzman (1988) *Ann. Neurol.* 23:138-144). Moreover, transgenic hAPP mice (Dodart, et al. (2002) *Nat. Neurosci.* 5:452-457; Kotilinek, et al. (2002) *J. Neurosci.* 22:6331-6335), which develop age-dependent

amyloid plaques and, most importantly, age-dependent memory dysfunction, show that within 24 hours of vaccination with monoclonal antibodies against Aβ memory loss can be reversed with no change in plaque levels. Such findings are not consistent with a mechanism for memory loss dependent on neuron death caused by amyloid fibrils.

Additional neurologically active molecules formed by Aβ self-assembly have been suggested. These molecules include soluble Aβ oligomers, also referred to as Aβ-derived diffusible ligands or ADDLs. Oligomers are metastable and form at low concentrations of Aβ1-42 (Lambert, et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:6448-6453). Aβ oligomers rapidly inhibit long-term potentiation (LTP), a classic experimental paradigm for memory and synaptic plasticity. As such, memory loss stems from synapse failure, prior to neuron death and synapse failure by Aβ oligomers, not fibrils (Hardy & Selkoe (2002) *Science* 297:353-356). Soluble oligomers have been found in brain tissue and are strikingly elevated in Alzheimer's Disease (Kayed, et al. (2003) *Science* 300:486-489; Gong, et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:10417-10422) and in hAPP transgenic mice Alzheimer's Disease models (Kotilinek, et al. (2002) *J. Neurosci.* 22:6331-6335; Chang, et al. (2003) *J. Mol. Neurosci.* 20:305-313).

A variety of Alzheimer's Disease treatment options have been suggested. Vaccine clinical trials have revealed that persons mounting a vigorous immune response to the vaccine exhibit cognitive benefit (Hock, et al. (2003) *Neuron* 38:547-554); however, frequency of CNS inflammation caused early termination of part of the trial (Birmingham & Frantz (2002) *Nat. Med.* 8:199-200). As an alternative to a vaccine, therapeutic antibodies that target ADDLs without binding monomers or fibrils have been suggested (Klein

(2002) *Neurochem. Int.* 41:345-352). ADDLs are highly antigenic, generating oligomer-selective polyclonal antibodies in rabbits at concentration of ~50 μg/mL (Lambert, et al. (2001) *J. Neurochem.* 79:595-605). Results from transgenic mice models also suggest that antibodies can be successful in reversing memory decline (Dodart, et al. (2002) *Nat. Neurosci.* 5:452-457; U.S. Patent Application Serial No. 11/194,989). Accordingly, there is a need in the art for ADDL-selective therapeutic antibodies for the prevention and treatment of Alzheimer's Disease. The present invention meets this need.

Summary of the Invention

The present invention is an isolated antibody, or fragment thereof, capable of differentially recognizing a multi-dimensional conformation of one or more Aβ-derived diffusible ligands. In particular, the antibody of the instant invention has a complementary determining region (CDR) of Arg-Xaa_1-Leu-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa_1 is Gln or Ala; Xaa_2 is Ser or Gly; Xaa_3 is Pro, Ala, Lys, Arg, or Thr; Xaa_4 is Lys or Arg; Xaa_5 is Gly, Ser, or Lys; Xaa_6 is Val, Thr, Ile or Arg. In particular embodiments, the antibody of the present invention is in admixture with a pharmaceutically acceptable carrier. In other embodiments, the antibody of the present invention is in a kit. Still other embodiments embrace an antibody having heavy and light chain variable region sequences as set forth in SEQ ID NO:108 and SEQ ID NO:112. An antibody having heavy and light chain sequences as set forth in SEQ ID NO:138 and SEQ ID NO:140 is also provided.

Methods for preventing binding of Aβ-derived diffusible ligands to a neuron and inhibiting assembly of

Aβ-derived diffusible ligands employing an antibody or antibody fragment which binds a multi-dimensional conformation of one or more Aβ-derived diffusible ligands are also provided.

The present invention further embraces a method for prophylactically or therapeutically treating a disease associated with Aβ-derived diffusible ligands using an antibody of the instant invention. Administration of an antibody of the invention can prevent binding of Aβ-derived diffusible ligands to a neuron thereby preventing or treating the disease associated with Aβ-derived diffusible ligands.

The present invention is also a method for identifying a therapeutic agent that prevents the binding of Aβ-derived diffusible ligands to a neuron. This method of the invention involves contacting a neuron with Aβ-derived diffusible ligands in the presence of an agent and using an antibody of the present invention to determine binding of the Aβ-derived diffusible ligands to the neuron in the presence of the agent.

The present invention also embraces a method for detecting Aβ-derived diffusible ligands in a sample and a method for diagnosing a disease associated with Aβ-derived diffusible ligands. Such methods involve contacting a sample with an antibody of the instant invention so that the Aβ-derived diffusible ligands can be detected and a disease associated with Aβ-derived diffusible ligands can be diagnosed.

Brief Description of the Drawings

Figure 1 shows the nucleic acid sequences for the heavy (Figure 1A) and light (Figure 1B) chain variable regions for murine anti-ADDL antibody 20C2. Lower case

letters indicate the antibody leader sequences and uppercase letters indicate antibody variable region sequences. The nucleotides coding for the complementary determining regions (CDRs) are underlined.

Figure 2 shows comparisons of heavy (Figure 2A) and light (Figure 2B) chain variable region amino acid sequences of murine antibody 20C2 and humanized antibodies, Hu20C2 (CDR grafted) and Hu20C2A3 (veneered). Sequences are presented as comparisons between the 20C2 mouse sequence, the most homologous human genomic sequence and the humanized sequences. Sequence differences in the frame regions between murine 20C2 and humanized Hu20C2A3 are in bold. Sequence differences in the underlined CDR regions between humanized Hu20C2A3 and murine 20C2 are in bold and indicated with an *. CDRs are underlined.

Figure 3 shows nucleic acid sequences for the heavy (Figures 3A and 3B) and light (Figure 3C) chain variable regions (HCVRs and LCVRs, respectively) for humanized anti-ADDL antibody Hu20C2 (CDR grafted). Two humanized versions of the Hu20C2 heavy chain were generated (HCVRA and HCVRB) that differ by one amino acid at position 24. In Hu20C2 HCVRA the human amino acid was used and in Hu20C2 HCVRB the mouse amino acid was used. Variable region sequences were cloned into full heavy and light chain antibody expression vectors.

Figure 4 shows the annotated amino acid sequences and nucleotide sequences of Hu20C2 humanized antibody in Fab phage-display vector pFab4. Amino acid sequence for heavy chain version A (Figure 4A), heavy chain version B (Figure 4B), and the light chain (Figure 4C) of Hu20C2 humanized antibody in Fab phage-display vector pFab4 are in italic and underlined regions are as indicated. Nucleotide sequence of heavy chain version A fused with the light

chain of Hu20C2 in pFab4 vector is shown in Figure 4D-4E with sequences encoding the Hu20C2 antibody sequences shown in lowercase.

Figure 5 depicts the design and primers employed in preparing two light chain CDR3 libraries, namely LC3-1 and LC3-2 (Figure 5A), and three heavy chain CDR3 libraries, namely 20C2B-39HC_3-1, 20C2B-39HC_3-2, and 20C2B-39HC_3-3 (Figure 5B), for respectively generating affinity matured Hu20C2 light and heavy chain CDR3s. Restriction endonuclease recognition sites used for cloning are indicated in italic. Uppercase indicates nucleic acids encoding antibody variable region sequences. Nucleic acids encoding CDRs are underlined. Biotin-labeled primers are indicated.

Figure 6 shows a comparison of the amino acid sequence of human antibody constant regions and the sequence of IgG2m4. The asterisk indicates a glycosylation site at Asn297. Regions of FcRn binding are indicated. Sequences in which IgG2m4 is different from IgG2 are underlined.

Figure 7 shows the amino acid (Figures 7A and 7C) and nucleotide (Figures 7B and 7D) sequences for the full IgG2m4 humanized heavy chain (Figures 7A and 7B) and humanized Kappa light chain (Figures 7C and 7D) for anti-ADDL antibody Hu20C2A3. Underlining indicates variable region sequences. The remaining sequences are constant region sequences.

Figure 8 shows interactions between Aβ40 monomer or ADDLs with Hu20C2A3 produced by two different systems, CHO (Figure 8A) or *Pichia* (Figure 8B), as determined by ELISA.

Figure 9 shows Hu20C2A3 inhibition of bADDL binding to primary hippocampal neurons.

Figure 10 shows fluorescent thermal melt analysis of Hu20C2A3.

Figure 11 shows plasma Aβx-40 levels (pM) of APP-YAC mice following intravenous injection of Hu20C2A3, irrelevant control or vehicle. Hu20C2A3 was prepared by stable transfection of CHO cells or *Pichia*. Aβx-40 was determined using a 4G8/G2-10 ELISA. ***, $p<0.001$ by Tukey-Kramer HSD post-hoc testing. Error bars = SEM; N=6/group.

Detailed Description of the Invention

Monoclonal antibodies, which differentially recognize multi-dimensional conformations of Aβ-derived diffusible ligands (*i.e.*, ADDLs), have now been generated. Antibodies of this invention are derived from the murine monoclonal antibody 20C2. Murine 20C2 is known in the art for exhibiting the following characteristics. Murine 20C2 is an IgG1 antibody which binds to both synthetic and endogenous ADDLs bound to cultured hippocampal cells. Furthermore, this antibody can block both endogenous and synthetic ADDL binding to cultured cells, abate the binding of biotinylated ADDLs (bADDLs) to neurons, and prevent tau phosphorylation. The core linear epitope for 20C2 is Glu-Phe-Arg-His-Asp-Ser (SEQ ID NO:1), corresponding to amino acid residues 3-8 of Aβ1-42, with a conformational epitope that is dependent upon elements from within residues 17-42 of Aβ, but only when assembled.

The instant antibodies are humanized and, in some embodiments affinity-matured derivatives of murine 20C2. Like the murine 20C2 antibody, the antibodies disclosed herein exhibit a high degree of selectivity for multi-dimensional conformations of ADDLs, with minimal detection of monomer Aβ peptides. Advantageously, the instant antibodies identify endogenous oligomers in Alzheimer's Disease brain slices and inhibit binding of bADDLs to neurons. Moreover, the instant antibodies provide a

significant and robust increase in plasma Aβx-40 levels, an increase in which is known to be associated with an ultimate lowering of brain Aβ. Accordingly, the antibodies of this invention find use in the prevention of ADDL binding to neurons and assembly of ADDLs and the treatment of ADDL-related diseases including Alzheimer's Disease.

Accordingly, the present invention is an isolated antibody that differentially recognizes one or more multi-dimensional conformations of ADDLs. An antibody of the instant invention is said to be isolated when it is present in the substantial absence of other biological macromolecules of the same type. Thus, an "isolated antibody" refers to an antibody which is substantially free of other antibodies; however, the molecule may include some additional agents or moieties which do not deleteriously affect the basic characteristics of the antibody (*e.g.*, binding specificity, neutralizing activity, etc.).

An antibody which is capable of specifically binding one or more multi-dimensional conformations of ADDLs, binds particular ADDLs derived from the oligomerization of Aβ1-42, but like murine 20C2 does not cross-react with other Aβ peptides, namely Aβ1-12, Aβ1-28, Aβ1-40, and Aβ12-28 as determined by western blot analyses; and preferentially bind ADDLs in solution. Specific binding between two entities generally refers to an affinity of at least 10^6, 10^7, 10^8, 10^9, or 10^{10} M^{-1}. Affinities greater than 10^8 M^{-1} are desired to achieve specific binding.

In particular embodiments, an antibody that is capable of specifically binding a multi-dimensional conformation of one or more ADDLs is also raised against (*i.e.*, an animal is immunized with) multi-dimensional conformations of ADDLs. In other embodiments, an antibody that is capable of specifically binding a multi-dimensional conformation of

one or more ADDLs is raised against a low n-mer-forming peptide such as Aβ1-42[Nle35-Dpro37].

The term "epitope" refers to a site on an antigen to which B and/or T cells respond or a site on a molecule against which an antibody will be produced and/or to which an antibody will bind. For example, an epitope can be recognized by an antibody defining the epitope.

A linear epitope is an epitope wherein an amino acid primary sequence comprises the epitope recognized. A linear epitope typically includes at least 3, and more usually, at least 5, for example, about 6 to about 10 amino acids in a unique sequence.

A conformational epitope, in contrast to a linear epitope, is an epitope wherein the primary sequence of the amino acids comprising the epitope is not the sole defining component of the epitope recognized (*e.g.*, an epitope wherein the primary sequence of amino acids is not necessarily recognized by the antibody defining the epitope). Typically a conformational epitope encompasses an increased number of amino acids relative to a linear epitope. With regard to recognition of conformational epitopes, the antibody recognizes a three-dimensional structure of the peptide or protein. For example, when a protein molecule folds to form a three-dimensional structure, certain amino acids and/or the polypeptide backbone forming the conformational epitope become juxtaposed enabling the antibody to recognize the epitope. Methods of determining conformation of epitopes include but are not limited to, for example, x-ray crystallography, two-dimensional nuclear magnetic resonance spectroscopy and site-directed spin labeling and electron paramagnetic resonance spectroscopy. See, for example, Epitope Mapping

Protocols in Methods in Molecular Biology (1996) Vol. 66, Morris (Ed.).

Aβ-derived diffusible ligands or ADDLs refer to soluble oligomers of amyloid β1-42 which are desirably composed of aggregates of less than eight or nine amyloid β1-42 peptides and are found associated with Alzheimer's Disease. This is in contrast to high molecular weight aggregation intermediates, which form stings of micelles leading to fibril formation.

As exemplified herein, the instant antibody binds or recognizes at least one multi-dimensional conformation of an ADDL. In particular embodiments, the instant antibody binds at least two, at least three, or at least four multi-dimensional conformations of an ADDL. Multi-dimensional conformations of ADDLs are intended to encompass dimers, trimers, tetramers pentamers, hexamers, heptamers, octamers, nonamers, decamers, etc. as defined by analysis via SDS-PAGE. Because trimer, tetramer, etc. designations can vary with the assay method employed (see, *e.g.*, Bitan, et al. (2005) *Amyloid* 12:88-95) the definition of trimer, tetramer, and the like, as used herein, is according to SDS-PAGE analysis. As such, the antibody of the instant invention has oligomer-specific characteristics. In particular embodiments, a multi-dimensional conformation of an ADDL is associated with a specific polypeptide structure which results in a conformational epitope that is recognized by an antibody of the present invention. In other embodiments, an antibody of the invention specifically binds a multi-dimensional conformation ADDL having a size range of approximately a trimer or tetramer, which have molecular weights in excess of >50 kDa.

In certain embodiments, in addition to binding to a multi-dimensional conformation, the instant antibody binds

to a selected linear epitope of amyloid β1-42. A linear epitope of an ADDLs is intended as a four, five, six or more amino acid residue peptide located in the N-terminal 10, 11, 12, 15 or 20 amino acid residues of amyloid β1-42. In particular embodiments, an antibody of the invention specifically binds to a linear epitope within residues 1-10, 1-8, 3-10, or 3-8 of amyloid β1-42. An exemplary linear epitope of amyloid β1-42 which is bound by a humanized antibody of the invention is amino acid residues Glu-Phe-Arg-His-Asp-Ser (SEQ ID NO:1).

While antibodies of the instant invention may have similar linear epitopes, such linear epitopes are not wholly indicative of the binding characteristics of the instant antibodies (*i.e.*, ability to block ADDL binding to neurons, prevent tau phosphorylation and inhibit ADDL assembly) because, as is well-known to the skilled artisan, the linear epitope may only correspond to a portion of the antigen's epitope (see, *e.g.*, Breitling and Dübel (1999) *In*: Recombinant Antibodies, John Wiley & Sons, Inc., NY, pg. 115). For example, murine 20C2 is known to bind assemblies of charge-inverted, truncated Aβ7-42 peptide, which lack the linear epitope for 20C2 (*i.e.*, amino acid residues 3-8) and contain a very different sequence corresponding to residues 7-16 of Aβ. Therefore, 20C2, as well as humanized derivatives thereof, bind to conformational epitopes that depend upon elements from within residues 17-42 of Aβ, but only when in a multidimensional conformation. The antibody of the instant invention can be distinguished from those of the art as being capable of differentially recognizing multi-dimensional ADDLs and accordingly differentially blocking ADDL binding to neurons, differentially preventing tau

phosphorylation and differentially inhibiting ADDL assembly.

An antibody, as used in accordance with the instant invention includes, but is not be limited to, monoclonal antibodies, and chimeric, human (*e.g.* isolated from B cells), humanized, neutralizing, bispecific or single chain antibodies thereof. In one embodiment, an antibody of the instant invention is monoclonal. For the production of antibodies, various hosts including goats, rabbits, chickens, rats, mice, humans, and others, can be immunized by injection with synthetic or natural ADDLs. Methods for producing antibodies are well-known in the art. See, *e.g.*, Kohler and Milstein ((1975) *Nature* 256:495-497) and Harlow and Lane (Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York (1988)).

Depending on the host species, various adjuvants can be used to increase the immunological response. Adjuvants used in accordance with the instant invention desirably augment the intrinsic response to ADDLs without causing conformational changes in the immunogen that affect the qualitative form of the response. Particularly suitable adjuvants include 3 De-O-acylated monophosphoryl lipid A (MPL™; RIBI ImmunoChem Research Inc., Hamilton, MT; see GB 2220211) and oil-in-water emulsions, such as squalene or peanut oil, optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute, et al. (1997) *N. Engl. J. Med.* 336:86-91), muramyl peptides (*e.g.*, N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (E-PE), N-acetylglucsaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-dipalmitoxy propylamide (DTP-DPP)), or other

bacterial cell wall components. Specific examples of oil-in-water emulsions include MF59 (WO 90/14837), containing 5% Squalene, 0.5% TWEEN™ 80, and 0.5% SPAN 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA); SAF containing 10% Squalene, 0.4% TWEEN™ 80, 5% PLURONIC®-blocked polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion; and RIBI™ adjuvant system (RAS) (Ribi ImmunoChem, Hamilton, MT) containing 2% squalene, 0.2% TWEEN™ 80, and one or more bacterial cell wall components such as monophosphoryllipid A, trehalose dimycolate (TDM), and cell wall skeleton (CWS).

Another class of adjuvants is saponin adjuvants, including ISCOMs (immunostimulating complexes) and ISCOMATRIX® (CSL Ltd., Parkville, Australia). Other suitable adjuvants include Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin, PLURONIC® polyols, polyanions, peptides, CpG (WO 98/40100), keyhole limpet hemocyanin, dinitrophenol, and cytokines such as interleukins (IL-1, IL-2, and IL-12), macrophage colony stimulating factor (M-CSF), and tumor necrosis factor (TNF). Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are particularly suitable.

An antibody to a multi-dimensional conformation ADDL is generated by immunizing an animal with ADDLs. Generally, ADDLs can be generated synthetically or by recombinant fragment expression and purification. Synthetic ADDLs can be prepared as disclosed herein or in accordance with the methods disclosed in U.S. Patent No. 6,218,506 or in co-

pending applications US Serial Nos. 60/621,776, 60/652,538, 60/695,528 and 60/695,526. Further, ADDLs can be fused with another protein such as keyhole limpet hemocyanin to generate an antibody against the chimeric molecule. The ADDLs can be conformationally constrained to form an epitope useful as described herein and furthermore can be associated with a surface for example, physically attached or chemically bonded to a surface in such a manner so as to allow for the production of a conformation which is recognized by the antibodies of the present invention.

Monoclonal antibodies to multi-dimensional conformations of ADDLs can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, et al. (1975) *Nature* 256:495-497; Kozbor, et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; Cole, et al. (1984) *Mol. Cell Biol.* 62:109-120).

In particular embodiments, the instant antibodies are humanized. Humanized or chimeric antibodies can be produced by splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity (see Morrison, et al. (1984) *Proc. Natl. Acad. Sci.* 81, 6851-6855; Neuberger, et al. (1984) *Nature* 312:604-608; Takeda, et al. (1985) *Nature* 314:452-454; Queen, et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:10029-10033; WO 90/07861). For example, a mouse antibody is expressed as the Fv or Fab fragment in a phage selection vector. The gene for the light chain (and in a parallel experiment, the gene for the heavy chain) is exchanged for a library of human antibody genes. Phage antibodies, which

still bind the antigen, are then identified. This method, commonly known as chain shuffling, provided humanized antibodies that should bind the same epitope as the mouse antibody from which it descends (Jespers, et al. (1994) *Biotechnology NY* 12:899-903). As an alternative, chain shuffling can be performed at the protein level (see, Figini, et al. (1994) *J. Mol. Biol.* 239:68-78).

Human antibodies can also be obtained using phage-display methods. See, *e.g.*, WO 91/17271 and WO 92/01047. In these methods, libraries of phage are produced in which members display different antibodies on their outer surfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to ADDLs. Human antibodies against ADDLs can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus. See, *e.g.*, WO 93/12227 and WO 91/10741. Human antibodies can be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Such antibodies generally retain the useful functional properties of the mouse antibodies. Human polyclonal antibodies can also be provided in the form of serum from humans immunized with an immunogenic agent. Optionally, such polyclonal antibodies can be concentrated by affinity purification using ADDLs as an affinity reagent.

As exemplified herein, humanized antibodies can also be produced by veneering or resurfacing of murine antibodies. Veneering involves replacing only the surface fixed region amino acids in the mouse heavy and light variable regions with those of a homologous human antibody

sequence. Replacing mouse surface amino acids with human residues in the same position from a homologous human sequence has been shown to reduce the immunogenicity of the mouse antibody while preserving its ligand binding. The replacement of exterior residues generally has little, or no, effect on the interior domains, or on the interdomain contacts. (See, *e.g.*, U.S. Patent No. 6,797,492).

Human or humanized antibodies can be designed to have IgG, IgD, IgA, IgM or IgE constant regions, and any isotype, including IgG1, IgG2, IgG3 and IgG4. In particular embodiments, an antibody of the invention is IgG or IgM, or a combination thereof. Other embodiments of the present invention embrace a constant region formed by selective incorporation of human IgG4 sequences into a standard human IgG2 constant region. An exemplary mutant IgG2 Fc is IgG2m4, set forth herein as SEQ ID NO:140. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains and light chains or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer. Techniques for the production of single chain antibodies are well-known in the art.

Exemplary humanized antibodies derivatives of murine 20C2 monoclonal antibody are provided herein by CDR grafting and veneering. Amino acid sequences for IgG2M4 heavy chain variable regions, as well as kappa light chain variable regions for humanized 20C2 (*i.e.*, Hu20C2A3) generated by veneering are presented in Figures 7A and 7C and set forth herein as SEQ ID NO:141 and SEQ ID NO:143.

Diabodies are also contemplated. A diabody refers to an engineered antibody construct prepared by isolating the binding domains (both heavy and light chain) of a binding antibody, and supplying a linking moiety which joins or

operably links the heavy and light chains on the same polypeptide chain thereby preserving the binding function (see, Holliger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444; Poljak (1994) *Structure* 2:1121-1123). This forms, in essence, a radically abbreviated antibody, having only the variable domain necessary for binding the antigen. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. These dimeric antibody fragments, or diabodies, are bivalent and bispecific. The skilled artisan will appreciate that any method to generate diabodies can be used. Suitable methods are described by Holliger, et al. (1993) *supra*, Poljak (1994) *supra*, Zhu, et al. (1996) *Biotechnology* 14:192-196, and U.S. Patent No. 6,492,123, incorporated herein by reference.

Fragments of an isolated antibody of the invention are also expressly encompassed by the instant invention. Fragments are intended to include Fab fragments, $F(ab')_2$ fragments, F(ab') fragments, bispecific scFv fragments, Fd fragments and fragments produced by a Fab expression library, as well as peptide aptamers. For example, $F(ab')_2$ fragments are produced by pepsin digestion of the antibody molecule of the invention, whereas Fab fragments are generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (see Huse, et al. (1989) *Science* 254:1275-1281). In particular embodiments, antibody fragments of the present invention are fragments of neutralizing antibodies which retain the variable region binding site thereof. Exemplary are $F(ab')_2$ fragments, F(ab') fragments, and Fab fragments. See

generally Immunology: Basic Processes (1985) 2nd edition, J. Bellanti (Ed.) pp. 95-97.

Peptide aptamers which differentially recognize multi-dimensional conformations of ADDLs can be rationally designed or screened for in a library of aptamers (*e.g.*, provided by Aptanomics SA, Lyon, France). In general, peptide aptamers are synthetic recognition molecules whose design is based on the structure of antibodies. Peptide aptamers consist of a variable peptide loop attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to that of an antibody (nanomolar range).

Exemplary nucleic acid sequences encoding heavy and light chain variable regions for use in producing antibody and antibody fragments of the instant invention are respectively disclosed herein in Figures 7B and 7D (*i.e.*, SEQ ID NOs:142 and 144). As will be appreciated by the skilled artisan, the heavy chain variable regions disclosed herein can be used in combination with any one of the light chain variable regions disclosed herein to generate antibodies with modified affinities, dissociate constants, epitopes and the like.

Antibodies or antibody fragments of the present invention can have additional moieties attached thereto. For example, a microsphere or microparticle can be attached to the antibody or antibody fragment, as described in U.S. Patent No. 4,493,825, the disclosure of which is incorporated herein by reference.

Moreover, particular embodiment embrace antibody or antibody fragments which are mutated and selected for increased antigen affinity, neutralizing activity (*i.e.*, the ability to block binding of ADDLs to neuronal cells or

the ability to block ADDL assembly), or a modified dissociation constant. Mutator strains of *E. coli* (Low, et al. (1996) *J. Mol. Biol.* 260:359-368), chain shuffling (Figini, et al. (1994) *supra*), and PCR mutagenesis are established methods for mutating nucleic acid molecules encoding antibodies. By way of illustration, increased affinity can be selected for by contacting a large number of phage antibodies with a low amount of biotinylated antigen so that the antibodies compete for binding. In this case, the number of antigen molecules should exceed the number of phage antibodies, but the concentration of antigen should be somewhat below the dissociation constant. Thus, predominantly mutated phage antibodies with increased affinity bind to the biotinylated antigen, while the larger part of the weaker affinity phage antibodies remains unbound. Streptavidin can then assist in the enrichment of the higher affinity, mutated phage antibodies from the mixture (Schier, et al. (1996) *J. Mol. Biol.* 255:28-43). Exemplary affinity-maturated light chain CDR3 amino acid sequences are disclosed herein (see Tables 6 and 7), with particular embodiments embracing a light chain CDR3 amino acid sequence of Xaa_1-Gln-Xaa_2-Thr-Arg-Val-Pro-Leu-Thr (SEQ ID NO:2), wherein Xaa_1 is Phe or Leu, and Xaa_2 is Ala or Thr. Affinity-maturated heavy chain CDR3 amino acid sequences are also provided herein. An exemplary heavy chain CDR3 amino acid sequence is set forth herein as Arg-Gln-Leu-Gly-Thr-Arg-Gly-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:3). The present invention also embraces derivatives of this CDR3, *e.g.*, Arg-Ala-Leu-Ser-Pro-Arg-Ser-Ile-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:4), Arg-Gln-Leu-Gly-Ala-Arg-Lys-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:5), Arg-Gln-Leu-Gly-Pro-Arg-Lys-Arg-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:6), Arg-Gln-Leu-Gly-Lys-Leu-Lys-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:7), or Arg-

Gln-Leu-Gly-Arg-Arg-Ser-Val-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:8), wherein differences with the Hu20C2A3 heavy chain CDR3 are underlined. In this regard, the present invention specifically embraces an anti-ADDL antibody having a CDR3 amino acid sequence of Arg-Xaa_1-Leu-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa_1 is Gln or Ala; Xaa_2 is Ser or Gly; Xaa_3 is Pro, Ala, Lys, Arg, or Thr; Xaa_4 is Lys or Arg; Xaa_5 is Gly, Ser, or Lys; Xaa_6 is Val, Thr, Ile or Arg

Other antibody derivatives encompassed within the scope of the present invention include any humanized antibody identical to Hu20C2A3's variable regions except with a one amino acid residue difference in the frame region of the light chain (*e.g.*, Leu-Pro-Val-Thr-Pro-Gly-Glu-Pro-Ala-Ser, SEQ ID NO:10).

For some therapeutic applications it may be desirable to reduce the dissociation of the antibody from the antigen. To achieve this, phage antibodies are bound to biotinylated antigen and an excess of unbiotinylated antigen is added. After a period of time, predominantly the phage antibodies with the lower dissociation constant can be harvested with streptavidin (Hawkins, et al. (1992) *J. Mol. Biol.* 226:889-96).

Various immunoassays including those disclosed herein can be used for screening to identify antibodies, or fragments thereof, having the desired specificity for multi-dimensional conformations of ADDLs. Numerous protocols for competitive binding (*e.g*, ELISA), latex agglutination assays, immunoradiometric assays, kinetics (*e.g.*, BIACORE™ analysis) using either polyclonal or monoclonal antibodies, or fragments thereof, are well-known in the art. Such immunoassays typically involve the measurement of complex formation between a specific

antibody and its cognate antigen. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes is suitable, but a competitive binding assay can also be employed. Such assays can also be used in the detection of multi-dimensional conformations of ADDLs in a sample.

An antibody or antibody fragment can also be subjected to other biological activity assays, *e.g.*, displacement of ADDL binding to neurons or cultured hippocampal cells or blockade of ADDL assembly, in order to evaluate neutralizing or pharmacological activity and potential efficacy as a prophylactic or therapeutic agent. Such assays are described herein and are well-known in the art.

Antibodies and fragments of antibodies can be produced and maintained as hydridomas or alternatively recombinantly produced in any well-established expression system including, but not limited to, *E. coli*, yeast (*e.g.*, *Saccharomyces* spp. and *Pichia* spp.), baculovirus, mammalian cells (*e.g.*, myeloma, CHO, COS), plants, or transgenic animals (Breitling and Dübel (1999) *In*: Recombinant Antibodies, John Wiley & Sons, Inc., NY, pp. 119-132). Antibodies and fragments of antibodies can be isolated using any appropriate methods including, but not limited to, affinity chromatography, immunoglobulins-binding molecules (*e.g.*, proteins A, L, G or H), tags operatively linked to the antibody or antibody fragment (*e.g.*, His-tag, FLAG®-tag, Strep tag, c-myc tag) and the like. See, Breitling and Dübel (1999) *supra*.

Antibodies and antibody fragments of the instant invention have a variety of uses including, diagnosis of diseases associated with accumulation of ADDLs, blocking or inhibiting binding of ADDLs to neuronal cells, blocking ADDL assembly, prophylactically or therapeutically treating

a disease associated with ADDLs, identifying therapeutic agents that prevent binding of ADDLs to neurons, and preventing the phosphorylation of tau protein at Ser202/Thr205.

Antibody and antibody fragments of the instant invention are also useful in a method for blocking or inhibiting binding of ADDLs to neuronal cells. This method of the invention is carried out by contacting a neuron, *in vitro* or *in vivo*, with an antibody or antibody fragment of the present invention so that binding of ADDLs to the neuron is blocked. In particular embodiments, an antibody or antibody fragment of the instant invention achieves at least a 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 97% decrease in the binding of ADDLs as compared to binding of ADDLs in the absence of the antibody or antibody fragment. The degree to which an antibody can block the binding of ADDLs to a neuron can be determined in accordance with the methods disclosed herein, *i.e.*, immunocytochemistry or cell-based alkaline phosphatase assay or any other suitable assay. Antibodies particularly useful for decreasing binding of ADDLs to neuronal cells include anti-ADDL antibodies having a CDR3 amino acid sequence set forth in SEQ ID NO:9, as well as derivatives and fragments thereof.

Antibody and antibody fragments of the instant invention are further useful in a method for blocking or inhibiting assembly of ADDLs. This method involves contacting a sample containing amyloid β 1-42 peptides with an antibody or antibody fragment of the instant invention so that ADDL assembly is inhibited. The degree to which an antibody can block the assembly of ADDLs can be determined in accordance with the methods disclosed herein, *i.e.*, FRET or fluorescence polarization or any other suitable assay.

Antibodies particularly useful for blocking the assembly of ADDLs include anti-ADDL antibodies having a CDR3 amino acid sequence set forth in SEQ ID NO:9, as well as derivatives and fragments thereof.

Antibodies disclosed herein are also useful in methods for preventing the phosphorylation of tau protein at Ser202/Thr205. This method involves contacting a sample containing tau protein with an antibody or antibody fragment of the instant invention so that binding of ADDLs to neurons is blocked thereby preventing phosphorylation of tau protein. The degree to which an antibody can prevent the phosphorylation of tau protein at Ser202/Thr205 can be determined in accordance with the methods disclosed herein or any other suitable assay.

Blocking or decreasing binding of ADDLs to neurons, inhibiting assembly of ADDLs, and preventing the phosphorylation of tau protein at Ser202/Thr205 all find application in methods of prophylactically or therapeutically treating a disease associated with the accumulation of ADDLs. Accordingly, the present invention also embraces the use of an antibody or antibody fragment of the instant invention to prevent or treat a disease associated with the accumulation of ADDLs (*e.g.* Alzheimer's or similar memory-related disorders). Evidence in the art indicates that elevated levels of Aβ, but not necessarily aggregated plaque, are causative for Alzheimer's Disease-associated dementia and subsequent tau abnormalities. Aβ-derived diffusible ligands are directly implicated in neurotoxicity associated with Alzheimer's Disease. The art indicates that ADDLs are elevated in transgenic mice and Alzheimer's Disease patients and modulate functional activity associated with mnemonic processes in animal models. Thus, removing this form of Aβ could provide relief

from the neurotoxicity associated with Alzheimer's Disease. As such, treatment with the instant antibody, which reduces central nervous system ADDL load, could prove efficacious for the treatment of Alzheimer's Disease. Patients amenable to treatment include individuals at risk of disease but not exhibiting symptoms, as well as patients presently exhibiting symptoms. In the case of Alzheimer's Disease, virtually anyone is at risk of suffering from Alzheimer's Disease if he or she lives long enough. Therefore, the antibody or antibody fragments of the present invention can be administered prophylactically to the general population without the need for any assessment of the risk of the subject patient. The present methods are especially useful for individuals who have a known genetic risk of Alzheimer's Disease. Such individuals include those having relatives who have been diagnosed with the disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk for Alzheimer's Disease include mutations in the APP gene, particularly mutations at position 717 and positions 670 and 671 referred to as the Hardy and Swedish mutations respectively. Other markers of risk are mutations in the presenilin genes, PS1 and PS2, and ApoE4, family history of Alzheimer's Disease, hypercholesterolemia or atherosclerosis. Individuals presently suffering from Alzheimer's Disease can be recognized from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have Alzheimer's Disease. These include measurement of CSF tau and Aβ1-42 levels. Individuals suffering from Alzheimer's Disease can also be diagnosed by ADRDA criteria or the method disclosed herein.

In asymptomatic patients, treatment can begin at any age (*e.g.*, 10, 20, 30 years of age). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70 years of age. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying for the presence of ADDLs over time.

In therapeutic applications, a pharmaceutical composition or medicament containing an antibody or antibody fragment of the invention is administered to a patient suspected of, or already suffering from such a disease associated with the accumulation of ADDLs in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease. In prophylactic applications, a pharmaceutical composition or medicament containing an antibody or antibody fragment of the invention is administered to a patient susceptible to, or otherwise at risk of, a disease associated with the accumulation of ADDLs in an amount sufficient to achieve passive immunity in the patient thereby eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In some methods, administration of agent reduces or eliminates myocognitive impairment in patients that have not yet developed characteristic Alzheimer's pathology. In particular embodiments, an effective amount of an antibody or antibody fragment of the invention is an amount which achieves at least a 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 97% decrease in the binding of ADDLs to

neurons in the patient as compared to binding of ADDLs in the absence of treatment. As such, impairment of long-term potentiation/memory formation is decreased.

Effective doses of the compositions of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but nonhuman mammals such as dogs or transgenic mammals can also be treated.

Treatment dosages are generally titrated to optimize safety and efficacy. For passive immunization with an antibody or antibody fragment, dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight are suitable. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. In some methods, two or more antibodies of the invention with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibodies are usually administered on multiple occasions, wherein intervals between single dosages can be weekly, monthly or yearly. An exemplary treatment regime entails subcutaneous dosing, once biweekly or monthly. Advantageously, subcutaneous administration has been found to reduce the flu-like symptoms associated with intravenous infusions (Lundin, et al. (2002) Blood 100:768-773). Intervals can also be irregular as indicated by measuring blood levels of antibody to ADDLs in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μg/mL and in some methods

25-300 μg/mL. Alternatively, the antibody or antibody fragment can be administered as a sustained-release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human and humanized antibodies have longer half-lives than chimeric antibodies and nonhuman antibodies. As indicated above, dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Antibody and antibody fragments of the instant invention can be administered as a component of a pharmaceutical composition or medicament. Pharmaceutical compositions or medicaments generally contain the active therapeutic agent and a variety of other pharmaceutically acceptable components. See Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, editor, 20th ed. Lippincott Williams & Wilkins: Philadelphia, PA, 2000. The preferred form depends on the intended mode of administration and therapeutic application. Pharmaceutical compositions can contain, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human

administration. Diluents are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution.

Pharmaceutical compositions can also contain large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex-functionalized SEPHAROSE™, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes).

Administration of a pharmaceutical composition or medicament of the invention can be carried out via a variety of routes including, but not limited to, oral, topical, pulmonary, rectal, subcutaneous, intradermal, intranasal, intracranial, intramuscular, intraocular, or intra-articular injection, and the like. The most typical route of administration is intravenous followed by subcutaneous, although other routes can be equally effective. Intramuscular injection can also be performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example, intracranial injection. In some embodiments, an antibody or antibody fragment is injected directly into the cranium. In other embodiments, antibody or antibody fragment is administered as a sustained-release composition or device, such as a MEDIPAD™ device.

For parenteral administration, antibody or antibody fragments of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a

pharmaceutical carrier that can be a sterile liquid such as water, oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are suitable liquid carriers, particularly for injectable solutions. Antibodies can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained-release of the active ingredient.

An exemplary composition contains the instant antibody or antibody fragment formulated as a sterile, clear liquid at a concentration of at least 10 mg/ml in isotonic buffered saline (10 mM histidine, 150 mM sodium chloride, 0.01% (w/v) POLYSORBATE 80, pH 6.0). An exemplary antibody formulation is filled as a single dose, 0.6 ml glass vials filled with 3.3 ml of solution per vial. Each vial is stopped with a TEFLON-coated stopper and sealed with an aluminum cap.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced delivery.

For suppositories, binders and carriers include, for example, polyalkylene glycols or triglycerides; such suppositories can be formed from mixtures containing the

active ingredient in the range of 0.5% to 10%, or more desirably 1%-2%.

Oral formulations include excipients, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained-release formulations or powders and contain 10%-95% of active ingredient, or more suitably 25%-70%.

Topical application can result in transdermal or intradermal delivery. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxins (see Glenn, et al. (1998) *Nature* 391:851). Co-administration can be achieved by using the components as a mixture or as linked molecules obtained by chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a skin path or using transferosomes (Paul, et al. (1995) *Eur. J. Immunol.* 25:3521-24; Cevc, et al. (1998) *Biochem. Biophys. Acta* 1368:201-15).

An antibody or antibody fragment of the invention can optionally be administered in combination with other agents that are at least partly effective in treatment of amyloidogenic disease. For example, the instant antibody can be administered with existing palliative treatments for Alzheimer's Disease, such as acetylcholinesterase inhibitors such as ARICEPT™, EXELON™, and REMINYL™ and, the NMDA antagonist, NAMENDA™. In addition to these approved treatments, the instant antibody can be used to provide synergistic/additive benefit for any of several approaches currently in development for the treatment of Alzheimer's

Disease, which include without limitation, inhibitors of Aβ production and aggregation.

Antibody and antibody fragments of the instant invention also find application in the identification of therapeutic agents that prevent the binding of ADDLs to neurons (*e.g.*, a hippocampal cell) thereby preventing downstream events attributed to ADDLs. Such an assay is carried out by contacting a neuron with ADDLs in the presence of an agent and using an antibody of antibody fragment of the invention to determine binding of the ADDLs to the neuron in the presence of the agent. As will be appreciated by the skilled artisan, an agent that blocks binding of ADDLs to a neuron will decrease the amount of ADDLs bound to the neuron as compared to a neuron which has not been contacted with the agent; an amount which is detectable in an immunoassay employing an antibody or antibody fragment of the instant invention. Suitable immunoassays for detecting neuronal-bound ADDLs are disclosed herein.

Agents which can be screened using the method provided herein encompass numerous chemical classes, although typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Agents encompass functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The agents often contain cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Agents can also be found among biomolecules including peptides, antibodies, saccharides,

fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Agents are obtained from a wide variety of sources including libraries of natural or synthetic compounds.

A variety of other reagents such as salts and neutral proteins can be included in the screening assays. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, and the like can be used. The mixture of components can be added in any order that provides for the requisite binding.

Agents identified by the screening assay of the present invention will be beneficial for the treatment of amyloidogenic diseases and/or tauopathies. In addition, it is contemplated that the experimental systems used to exemplify these concepts represent research tools for the evaluation, identification and screening of novel drug targets associated with amyloid beta induction of tau phosphorylation.

The present invention also provides methods for detecting ADDLs and diagnosing a disease associated with accumulation of ADDLs using an antibody or antibody fragment of the instant invention. A disease associated with accumulation of ADDLs is intended to include any disease wherein the accumulation of ADDLs results in physiological impairment of long-term potentiation/memory formation. Diseases of this type include, but are not limited to, Alzheimer's Disease and similar memory-related disorders.

In accordance with these methods, a sample from a patient is contacted with an antibody or antibody fragment of the invention and binding of the antibody or antibody fragment to the sample is indicative of the presence of

ADDLs in the sample. As used in the context of the present invention, a sample is intended to mean any bodily fluid or tissue which is amenable to analysis using immunoassays. Suitable samples which can be analyzed in accordance with the methods of the invention include, but are not limited to, biopsy samples and fluid samples of the brain from a patient (*e.g.*, a mammal such as a human). For *in vitro* purposes (*e.g.*, in assays monitoring oligomer formation), a sample can be a neuronal cell line or tissue sample. For diagnostic purposes, it is contemplated that the sample can be from an individual suspected of having a disease associated with accumulation of ADDLs or from an individual at risk of having a disease associated with accumulation of ADDLs, *e.g.*, an individual with a family history which predisposes the individual to a disease associated with accumulation of ADDLs.

Detection of binding of the antibody or antibody fragment to ADDLs in the sample can be carried out using any standard immunoassay (*e.g.*, as disclosed herein), or alternatively when the antibody fragment is, e.g., a peptide aptamer, binding can be directly detected by, for example, a detectable marker protein (*e.g.*, β-galactosidase, GFP or luciferase) fused to the aptamer. Subsequently, the presence or absence of the ADDL-antibody complex is correlated with the presence or absence, respectively, of ADDLs in the sample and therefore the presence or absence, respectively, of a disease associated with accumulation of ADDLs. It is contemplated that one or more antibodies or antibody fragments of the present invention can be used in conjunction with current non-invasive immuno-based imaging techniques to greatly enhance detection and early diagnosis of a disease associated with accumulation of ADDLs.

To facilitate diagnosis the present invention also pertains to a kit for containing an antibody or antibody fragment of the instant invention. The kit includes a container holding one or more antibody or antibody fragments which recognizes multi-dimensional conformation of ADDLs and instructions for using the antibody for the purpose of binding to ADDLs to form an antibody-antigen complex and detecting the formation of the antibody-antigen complex such that the presence or absence of the antibody-antigen complex correlates with presence or absence of ADDLs in the sample. Examples of containers include multiwell plates which allow simultaneous detection of ADDLs in multiple samples.

The invention is described in greater detail by the following non-limiting examples.

Example 1: General Materials and Methods

ADDL Preparation. ADDLs in F12 medium (Biosource, Camarillo, CA) were prepared from Aβ1-42 in accordance with established methods (Lambert, et al. (2001) *supra*). Briefly, Aβ1-42 peptide (American Peptide Co., Sunnyvale, CA or California Peptide Research, Inc., Napa, CA) was weighed and placed in a glass vial capable of holding a sufficient quantity of HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) to achieve a peptide concentration of 10 mg/mL. HFIP was added to the dry peptide, the vial was capped and gently swirl to mix, and the peptide/HFIP solution was stored at room temperature for at least one hour. Aliquots (50 or 100 μL, 0.5 or 1.0 mg, respectively) of peptide solution was dispensed into a series of 1.5 mL conical centrifuge tubes. The tubes were placed in a SPEEDVAC® overnight to remove the HFIP. Tubes containing the dried

peptide film were capped and stored at -70°C in a sealed container with dessicant.

Prior to use, the Aβ1-42 peptide film was removed from -70°C storage and allowed to warm to room temperature. Fresh DMSO (44 µL/mg of peptide film; 5 mM) was added and the peptide/DMSO mixture was incubated on a vortex mixer at the lowest possible speed for ten minutes. F12 media (2 mL/mg peptide) was dispensed into each tube of DMSO/peptide and the tube was capped and mixed by inversion. The 100 µM preparation was stored at 2-8°C for eighteen to twenty four hours. The samples were centrifuged at 14,000 x g for ten minutes at 2-8°C. The supernatant was transferred to a fresh tube and stored at 2-8°C until used.

Biotinylated ADDL preparations (bADDLs) were prepared in the same manner as described above for ADDL preparations using 100% *N*-terminal biotinylated amyloid beta peptide (American Peptide Company, Sunnyvale, CA).

Monomer Preparation. HFIP dry down preparations of amyloid beta (1-40) peptide (Aβ1-40) were prepared as outlined for Aβ(1-42) peptide. The peptide film was dissolved in 2 mL of 25 mM borate buffer (pH 8.5) per mg of peptide, divided into aliquots, and frozen at -70°C until used.

Primary Neurons. Primary hippocampal cultures were prepared from frozen, dissociated neonatal rat hippocampal cells (Cambrex, Corp., East Rutherford, NJ) that were thawed and plated in 96-well COSTAR® plates at a concentration of 20,000 cells per well. The cells were maintained in NEUROBASAL™ media without L-glutamine (GIBCO-BRL™, Gaithersburg, MD) and supplemented with B27 (GIBCO-BRL™, Gaithersburg, MD) for a period of two weeks and then used for binding studies.

Immunocytochemistry. Immunocytochemistry was performed according to established methods (Lambert, et al. (2001) *supra*), except the secondary antibodies were conjugated to ALEXAFLUOR® 588 (Molecular Probes, Eugene, OR). Antibodies and ADDLs were preincubated for 1 hour at room temperature, at a molar ratio of 1:4 antibody:ADDL before application to the 21-day hippocampal cell culture. For endogenous ADDLs, human brain protein (prepared as in Lambert, et al. (2001) *supra*) was incubated with cells for 1 hour before the cells were washed, fixed, and visualized as above.

Lightly fixed frozen sections (4% paraformaldehyde at 4°C for 30 hours and cryoprotected in 40 µm sucrose) from Alzheimer's Disease and control hippocampus were incubated with antibody (1:1000 in phosphate-buffered saline (PBS)) overnight at 4°C. After removal of antibody, sections were washed 3 times with PBS and incubated with secondary antibody at room temperature. Binding was then visualized with DAB (SIGMA™, St. Louis, MO). Sections were then counterstained with hematoxylin, mounted, and imaged on a NIKON® ECLIPSE® E600 light microscope with a SPOT™ INSIGHT™ digital video camera (v. 3.2).

ELISA. Polyclonal anti-ADDLs IgG (M90/1; Bethyl Laboratories, Inc., Montgomery, TX) was plated at 0.25 mg/well on IMMULON™ 3 REMOVAWELL™ strips (Dynatech Labs, Chantilly, VA) for 2 hours at room temperature and the wells blocked with 2% BSA in TBS. Samples diluted with 1% BSA in F12 were added to the wells, allowed to bind for 2 hours at 4°C, and washed 3X with BSA/TBS at room temperature. Monoclonal antibodies diluted in BSA/TBS were incubated for 90 minutes at room temperature and detected with a VECTASTAIN® ABC kit to mouse IgG. The HRP label was visualized with BIO-RAD® peroxidase substrate and read at 405 nm on a Dynex MRX-TC microplate reader.

Example 2: Isolation of Mouse Antibody Variable Region Sequences

The cDNAs coding for the variable domains of the 20C2 mouse antibody were cloned and sequenced following a polymerase chain reaction (PCR) using specially designed primers that hybridize to the 5'-ends of the mouse constant regions and to the murine leader sequences upstream of the V regions. This ensured that the mouse variable region sequences obtained were complete and accurate. In short, mRNA was extracted from mouse hybridoma cell lines using the QIAGEN® OLIGOTEX® Direct mRNA Mini Kit and subsequently converted to cDNA using a first-strand cDNA synthesis kit. The cDNA was then used as template in PCR reactions to obtain the antibody variable region sequences.

To obtain the light chain variable region sequence, eleven independent PCR reactions were set up using each of the eleven light chain 5′ PCR primers (MKV-1 to MKV-11) and the 3′ PCR primer MKC-1 (Table 1).

TABLE 1

5′ Primer	Sequence	SEQ ID NO:
MKV-1	GAT CTC TAG ATG AAG ATT GCC TGT TAG GCT GTT GGT GCT G	11
MKV-2	GAT CTC TAG ATG GAG WCA GAC ACA CTC CTG YTA TGG GTG	12
MKV-3	GAT CTC TAG ATG AGT GTG CTC ACT CAG GTC CTG GSG TTG	13
MKV-4	GAT CTC TAG ATG AGG RCC CCT GCT CAG WTT YTT GGM WTC TTG	14
MKV-5	GAT CTC TAG ATG GAT TTW CAG GTG CAG ATT WTC AGC TTC	15
MKV-6	GAT CTC TAG ATG AGG TKC YYT GYT SAY CTY CTC TGR GG	16
MKV-7	GAT CTC TAG ATG GGC WTC AAA GAT GGA GTC ACA KWY YCW GG	17
MKV-8	GAT CTC TAG ATG TGG GGA YCT KTT TYC MMT TTT TCA ATG	18
MKV-9	GAT CTC TAG ATG GTR TCC WCA SCT CAG TTC CTT G	19
MKV-10	GAT CTC TAG ATG TAT ATA TGT TTG TTG TCT ATT TCT	20
MKV-11	GAT CTC TAG ATG GAA GCC CCA GCT CAG CTT CTC TTC C	21
3′ Primer	Sequence	SEQ ID NO:
MKC-1	GAT CGA GCT CAC TGG ATG GTG GGA AGA TGG	22

Underlined and italic sequences denote *Xba*I and *Sac*I restriction sites, respectively. W = A or T, M = A or C, K = G or T, Y = C or T, and R = A or G.

To obtain the heavy chain variable region sequences twelve independent PCR reactions were set up using each of the twelve heavy chain 5′ PCR primers (MHV-1 to MHV-12) and the appropriate isotype specific 3′ primer (MHCG-1, MHCG-2A, MHCG-2B, MHCG-3) (Table 2).

TABLE 2

5′ Primer	Sequence	SEQ ID NO:
MHV-1	GAT CTC TAG ATG AAA TGC AGC TGG GGC ATS TTC TTC	23
MHV-2	GAT CTC TAG ATG GGA TGG AGC TRT ATC ATS YTC TT	24
MHV-3	GAT CTC TAG ATG AAG WTG TGG TTA AAC TGG GTT TTT	25
MHV-4	GAT CTC TAG ATG RAC TTT GGG YTC AGC TTG RTT T	26
MHV-5	GAT CTC TAG ATG GGA CTC CAG GCT TCA ATT TAG TTT TCC TT	27
MHV-6	GAT CTC TAG ATG GCT TGT CYT TRG SGC TRC TCT TCT GC	28
MHV-7	GAT CTC TAG ATG GRA TGG AGC KGG RGT CTT TMT CTT	29
MHV-8	GAT CTC TAG ATG AGA GTG CTG ATT CTT TTG TG	30
MHV-9	GAT CTC TAG ATG GMT TGG GTG TGG AMC TTG CTT ATT CCT G	31
MHV-10	GAT CTC TAG ATG GGC AGA CTT ACC ATT CTC ATT CCT G	32
MHV-11	GAT CTC TAG ATG GAT TTT GGG CTG ATT TTT TTT ATT G	33
MHV-12	GAT CTC TAG ATG ATG GTG TTA AGT CTT CTG TAC CTG	34
3′ Primer	Sequence	SEQ ID NO:
MHCG-1	GCATC *GAG CTC* CAG TGG ATA GAC AGA TGG GGG	35
MHCG-2A	GCATC *GAG CTC* CAG TGG ATA GAC CGA TGG GGG	36
MHCG-2B	GCATC GAG CTC CAG TGG ATG AGC TGA TGG GGG	37
MHCG-3	GCATC *GAG CTC* CAA GGG ATA GAC AGA TGG GGC	38

Underlined and italic sequences denote *Xba*I and *Sac*I restriction sites, respectively. W = A or T, M = A or C, K = G or T, Y = C or T, and R = A or G.

Each of the light chain PCR reactions contained 46 µL INVITROGEN™ PLATINUM® PCR Super Mix, 1.0 µL of one of the 100 µM 5′ primers (MKV-1 to MKV-11), 1.0 µL of the 100 µM 3′ primer (MKC-1), and 2.0 µL of hybridoma cDNA. Similar PCR reactions were employed to clone the mouse heavy chain variable region sequences. Reactions were placed in a DNA thermal cycler and, after an initial denaturation step at 97°C for 2.0 minutes, subjected to 30 cycles of: 95°C for 30 seconds, 55°C for 45 seconds, and 72°C for 90 seconds. Following the last cycle, a final extension step at 72°C for 10 minutes was employed. To determine which PCR reactions yielded product, 5 µL aliquots from each reaction

were separated on 1.5% (w/v) agarose/1X TAE buffer gels, containing 0.5 µg/mL ethidium bromide. PCR products from reactions that produced fragments of the expected size (420 to 500 bp) were then gel purified, digested with *Xba*I and *Sac*I and ligated into the *Xba*I and *Sac*I sites in the multicloning region of plasmid pNEB193 (New England Biolabs, Beverly, MA). Alternatively, PCR products were ligated directly into plasmid pCR®2.1 using the INVITROGEN™ TA CLONING® kit. Ligation products were then transformed into XL-1 cells and aliquots of the transformed *E. coli* were plated onto LB agar plates containing 50 µg/mL ampicillin and overlaid with 40 µL of X-Gal stock (50 mg/mL) and 40 µL IPTG (100 mM) solution for blue/white selection. Plates were incubated overnight at 37°C and potential clones were identified as white colonies. DNA from at least 24 independent clones for each PCR product were sequenced on both strands using universal forward and reverse primers for pNEB193 and pCR®2.1. The resulting sequences were then assembled into a contig to generate a consensus sequence for each antibody light and heavy chain variable region. Using this approach the sequences were determined for the light and heavy antibody variable regions of hybridoma 20C2 (Figures 1A-1B). The six complementarity-determining regions (CDRs), which form the structure complementary to the antigen, are underlined in Figures 1A-1B.

Example 3: Humanization of Mouse Anti-ADDL Antibody Variable Region Sequences

Mouse antibody heavy and light variable domain nucleic acids obtained from mouse hybridoma cell line 20C2 were humanized using a CDR grafting approach. It will be appreciated by those skilled in the art that humanization

of mouse antibody sequences can maximize the therapeutic potential of an antibody by improving its serum half-life and Fc effector functions thereby reducing the anti-globulin response.

Humanization by CDR grafting was carried out by selecting the human light and heavy chain variable regions from the NCBI protein database with the highest homology to the mouse variable domains. The mouse variable region sequences were compared to all human variable region sequences in the database using the protein-protein Basic Local Alignment Search Tool (BLAST). Subsequently, mouse CDRs were joined to the human framework regions and the preliminary amino acid sequence was analyzed. All differences between the mouse and human sequences in the framework regions were evaluated particularly if they were part of the canonical sequences for loop structure or were residues located at the VL/VH interface (O'Brien and Jones (2001) *In*: Antibody Engineering, Kontermann and Dubel (Eds.), Springer Laboratory Manuals). Framework regions were also scanned for unusual or rare amino acids in comparison to the consensus sequences for the human subgroup and for potential glycosylation sites. Wherein amino acid sequence differences existed between the mouse and human framework region sequences that were not found to be involved in canonical sequences, or located at the VL/VH interface, the human residue was selected at that position. Wherein a difference in a key residue existed, two versions of the variable region sequence were generated for evaluation. The CDR grafting strategy made the minimum number of changes to the human framework region so that good antigen binding was achieved while maintaining human framework regions that closely matched the sequence from a natural human antibody. The heavy chain and light chain

variable region amino acid sequences of the resulting humanized antibody generated by CDR grafting of murine 20C2 are shown in Figures 2A and 2B, respectively. This antibody is designated herein as Hu20C2.

Humanized sequences for 20C2 were also designed using a veneering strategy (See, *e.g.*, U.S. Patent No. 6,797,492). Humanization was carried out by selecting the human light and heavy chain variable regions from the NCBI protein database with the highest homology to the mouse variable domains, as well as to the closest human antibody germline family or families (see, Kabat, eta l. (1991) Sequences of proteins of immunological interest, 5th ed., U.S. Dept. Health and Human Services, NIH, Washington DC). The mouse variable region sequences were compared to all human variable region sequences in the database using protein-protein BLAST. The murine variable sequences and their closest human homologues were modeled to the closest crystallized human antibody as determined by computer modeling as practiced in the art. From the model of the murine VH and VL sequences, a surface area map was constructed, which dictated the solvent accessibility of the amino acids in the mouse heavy and light variable regions. To confirm the modeling, these exposed residues were compared position-by-position with known surface accessible residues (see, *e.g.*, Padlan (1994) *Mol. Immunol.* 31(3):169-217). A score was assigned for each residue in the sequence designating it as exposed, mostly exposed, partly buried, mostly buried and buried according to established methods (see, U.S. Patent No. 6,797,492, incorporated herein by reference in its entirety). Mouse framework residues that scored as exposed or mostly exposed and differed from the homologous human sequence were changed to the human residue at that position. The designed veneered

sequences retained the mouse CDRs, residues neighboring the CDRs, residues known be involved in canonical sequences, residues located at the VL/VH interface, and residues at the N-terminal sequences of the mouse heavy and light chain. The N-terminal sequences are known to be contiguous with the CDR surface and are potentially involved in ligand binding. Once the veneered sequences were finalized they were remodeled to look for are any potential obvious structural issues. A total of 12 and 9 amino acid residues were changed in the heavy chain and light chain frames, respectively. The heavy chain and light chain variable region amino acid sequences of the resulting humanized antibody generated by veneering of murine 20C2 are shown in Figures 2A and 2B, respectively. This antibody is designated herein as Hu20C2A3.

In comparison to 20C2, it is noted that the light chain substitutions resulting in Hu20C2A3, but not the heavy chain substitutions, are in common with Hu20C2 (Figure 2). In particular, heavy chain variable region CDR3 is unique to Hu20C2A3.

Once the humanized amino acid sequences were selected the sequences were reverse-translated to obtain the corresponding DNA sequence. The DNA sequences were codon-optimized using art-established methods (Lathe (1985) *J. Mol. Biol.* 183(1):1-12) and designed with flanking restriction enzyme sites for cloning into human antibody expression vectors. The nucleotide sequences encoding the light chain variable region and two versions of the heavy chain variable region for Hu20C2 are presented in Figures 3A-3C. The two heavy chain variable region versions differ by a single amino acid substitution at position 24; heavy chain variable region for version A of Hu20C2 is Phe at

position 24 and heavy chain variable region of version B of Hu20C2 is Leu at position 24.

Example 4: Affinity Maturation

Affinity maturation was carried out on the Hu20C2 antibody. Nucleic acid molecules encoding humanized Hu20C2 versions A and B variable heavy chain only, light chain only or heavy chain version A and light chain together were cloned in the Fab phage-display vector pFab4. Nucleic acid sequence analysis confirmed sequence and orientation in pFab4. The annotated Hu20C2 Fab sequences in pFab4 are presented in Figures 4A-4C and set forth herein as SEQ ID NO:116 for heavy chain version A, SEQ ID NO:117 for heavy chain version B, and SEQ ID NO:118 for the light chain. The nucleotide sequence for heavy chain version A and light chain together in the pFab4 vector is presented in Figures 4D-4E. These constructs were used in the Hu20C2 maturation program using art-established phage-displayed Fab library methods.

Light Chain Maturation. Two libraries were designed to mutate the nine wild-type amino acids of CDR3 of the light (kappa) chain of Hu20C2 (*i.e.*, Phe-Gln-Gly-Ser-Leu-Val-Pro-Leu-Thr; SEQ ID NO:39). These libraries were designated LC3-1 and LC3-2 representing light chain CDR3 sequences of Xaa-Xaa-Xaa-Xaa-Xaa-Val-Pro-Leu-Thr (SEQ ID NO:40) and Phe-Gln-Gly-Ser-Xaa-Xaa-Xaa-Xaa-Xaa (SEQ ID NO:41), respectively. Biotinylated reverse primers, 20C2LC3-1 (SEQ ID NO:123) and 20C2LC3-2 (SEQ ID NO:126), were used in combination with forward primer 20C2LC3F (SEQ ID NO:120) to generate the LC3-1 and LC3-2 libraries (see Figure 5A). Primers were purified by polyacrylamide gel electrophoresis, whereas the vector DNA was purified by gel electrophoresis and electroelution. The two light chain

libraries were designed to be randomly mutated. The final diversities of the three 10G5H6 LC_3 libraries were 4.76 x 10^8 and 7.45 x 10^8, respectively (Table 3). Sequence analysis of approximately 100 clones from the libraries showed 100% diversity of mutant clones at the designed amino acid positions.

TABLE 3

Characteristic	Light Chain Library	
	LC3-1	LC3-2
Vector	pFab3d20C2HS	pFab3d20C2HS
Number of Transformants	4.76×10^8	7.45×10^8
Library Diversity	$4.76 \times 10^8 \times 0.89 = 4.24\ 10^8$	$7.45 \times 10^8 \times 0.90 = 6.71\ 10^8$
Primary Library Volume	2 mL	2 mL
Primary Library Titer	2.13×10^{11}	*9.3×10^{10}

*Higher titers are achieved by concentration or phage rescue.

Soluble panning of the two light chain libraries against high molecular weight bADDL was completed. Briefly, four rounds of panning were carried out using biotinylated high molecular weight ADDL (bADDL). The first three rounds were carried out using approximately 1.5 μM antigen concentration (input = 1 x 10^{10} to 1 x 10^{11}). Upon completion of the third round, the outputs of the two libraries were combined and divided into three groups for analysis with 10 nM, 100 nM and approximately 1.5 μM antigen to increase panning stringency. As such, a total of 58 output plates were tested in phage ELISA assays, *i.e.*, two plates per library in the first round (a total of four plates), six plates per library in the second round (a total of 12 plates), eight plates for LC3-1 and 10 plates for LC3-2 libraries in the third round (a total of 18 plates) and

eight plates for each antigen concentration in the fourth round (a total of 24 plates).

Panning resulted in 1000 hits, 436 of which were sequenced (Table 4).

TABLE 4

Round	Antigen	Input	Output	% Recovery	ELISA Screen*	Sequenced
1[a]	1.6 μM	$2.13x10^{10}$	$7.3x10^{4}$	$3.42x10^{-6}$	0% (0/176)	0
2[a]	2.0 μM	$1.55x10^{11}$	$1.88x10^{5}$	$1.21x10^{-6}$	1.5% (8/528)	8
3[a]	1.1 μM	$1.80x10^{10}$	$7.8x10^{4}$	$4.3x10^{-6}$	5.8% (41/704)	41
1[b]	1.6 μM	$9.30x10^{9}$	$5.7x10^{4}$	$6.13x10^{-6}$	2.3% (7/176)	4
2[b]	2.0 μM	$1.23x10^{11}$	$1.07x10^{5}$	$8.7x10^{-7}$	4.5% (24/528)	24
3[b]	1.1 μM	$1.37x10^{10}$	$3.32x10^{5}$	$2.42x10^{-5}$	15% (134/880)	134
4[c]	1.1 μM	$3.0x10^{11}$	$1.37x10^{5}$	$4.6x10^{-7}$	39% (274/704)	-
4[c]	100 nM	$3.0x10^{11}$	$3.88x10^{5}$	$1.29x10^{-6}$	41% (290/704)	-
4[c]	10 nM	$3.0x10^{11}$	$1.6x10^{5}$	$5.3x10^{-7}$	32% (225/704)	225
				Total	1000/5104	436

[a]20C2 LC3-1 versus high molecular weight 10% bADDL.
[b]20C2 LC3-2 versus high molecular weight 10% bADDL.
[c]20C2 LC3-1 + 20C2 LC3-2 versus high molecular weight 10% bADDL.
*Hits per total number of colonies.

Sequence and frequency of highly enriched clones are presented in Table 5.

TABLE 5

Clone Designation	LC CDR3	SEQ ID NO:	Round 2	Round 3	Round 4	Total
Hu20C2LC	FQGSLVPLT	39	6	15	14	35
SJ-p1-31	ADTTHVPLT	42		1	2	3
SJ-p1-14	AHSTFVPLT	43	1	1	2	4
4P2-12-E3	AQASFVPLT	44			2	2
SJ-p1-38	AQATKVPLT	45		1	1	2
4P3-59	AQSSKVPLT	46			2	2
SJ-p2-14	AQSTLVPLT	47		1	2	3
4P3-11	FAASSVPLT	48			2	2
4P3-1	FESTYVPLT	49			2	2
SJ-p2-10	FESSRVPLT	50		1	1	2
SJ-p2-11	FNATWVPLT	51		2		2

SJ-p2-60	FQASRVPLT	52		1	5	6
SJ-p1-18	FQATRVPLT	53		1	5	6
SJ-p3-51	FQGSFIGLS	54	1		1	2
SJ-p3-16	FQGSFIPGT	55		2	3	5
SJ-p8-8F	FQGSFLPPS	56		1	1	2
SJ-p3-26	FQGSFLPQL	57	1	2		3
SJ-p3-15	FQGSLFPPV	58	1	2		3
SJ-p2-70	FQGSLFSPS	59	1	5		6
SJ-p3-24	FQGSRIPIS	60		1	1	2
SJ-p3-33	FQGSRLPVS	61		2	3	5
SJ-p3-14	FQGSRVPLV	62		2	1	3
SJ-p2-1F	FQSSFVPLT	63		6	8	14
4P1-22	FQSSRVPLT	64			15	15
SJ-p2-44	GQTTLVPLT	65		1	3	4
SJ-p1-56	HESTLVPLT	66		2	1	3
4P1-40	HQSSKVPLT	67			4	4
SJ-p2-20	IQTSLVPLT	68		2		2
SJ-p1-41	IQAALVPLT	69		1	1	2
SJ-p2-13	LQSSFVPLT	70	1	4		5
4P1-26	LETSRVPLT	71			3	3
SJ-p1-33	LASSHVPLT	72		2	1	3
SJ-p2-27	LNSTTVPLT	73		2	4	6
SJ-p2-62	LQSKSVPLT	74		2		2
4P2-26-E5	LQSVRVPLT	75			3	3
4P1-32	LQSSLVPLT	76			5	5
SJ-p2-37	LQTGRVPLT	77		2	2	4
SJ-p2-64	LQTSFVPLT	78		3		3
4P1-20	LQTSNVPLT	79			5	5
SJ-p2-39	LQTTRVPLT	80		2	6	8
SJ-p2-52	LSSTFVPLT	81		3	1	4
SJ-p2-6L	LSSTHVPLT	82		2	1	3
4P1-77	LTSSAVPLT	83			2	2
SJ-p1-59	LVSSLVPLT	84		2		2
SJ-p2-23	METANVPLT	85		2		2
SJ-p1-9M	MQSSFVPLT	86		1	3	4
SJ-p2-28	MQSSLVPLT	87		1	2	3
SJ-p1-21	MQTSKVPLT	88		1	1	2
4P1-17	SQARMVPLT	89			3	3
SJ-p2-66	SQASRVPLT	90		1	2	3
SJ-p1-49	TQSTQVPLT	91		2	1	3
SJ-p2-24	VCATFVPLT	92		1	1	2
4P1-41	VQSSAVPLT	93			2	2
SJ-p2-51	VQTSLVPLT	94		12	31	43
4P1-64	VQTSVVPLT	95			3	3
SJ-p2-55	VQTTAVPLT	96		2		2
SJ-p1-25	LQTARVPLT	97		1	3	4

Fab fragments from the 10 top clones based on enrichment frequency were prepared and a total of 15 clones were converted into IgG1 humanized A version and two clones, 20C2-6 and 20C2-8, were converted to IgG1 humanized

B version. K_D values for these clones were measured by BIACORE™ using biotin-Aβ1-20 (Table 6) and bADDL (Table 7) as antigens. Dramatic improvements in affinity were observed as compared to parental humanized 20C2A and 20C2B, as well as mouse 20C2 antibodies. In particular, low nanomolar to sub-picomolar K_Ds were achieved with a light chain CDR3 of the sequence Xaa_1-Gln-Xaa_2-Thr-Arg-Val-Pro-Leu-Thr (SEQ ID NO:2), wherein Xaa_1 is Phe or Leu, and Xaa_1 is Ala or Thr. Moreover, a comparison between K_D values obtained with BIACORE™ using biotin-Aβ1-20 and bADDL further demonstrates that anti-ADDL antibodies such as Hu20C2 preferentially bind multi-dimensional conformations of ADDLs over monomeric Aβ peptides.

TABLE 6

Name	Clone	LC-CDR3	SEQ ID NO:	K_D (Biotin-Aβ1-20)		
				Fab	IgG1#1	IgG1#2
20C2-1A	SJ-p2-60	FQASRVPLT	52	91 nM	1.2 nM	--
20C2-2A	SJ-p1-18	FQATRVPLT	53	28 nM	686 pM	2 nM
20C2-3A	SJ-p3-16	FQGSFIPGT	55	--	1.7 nM	--
20C2-5A	SJ-p2-1F	FQSSFVPLT	63	41 nM	912 pM	1.5 nM
20C2-6A	4P1-22	FQSSRVPLT	64	18 nM	544 pM	714 pM
20C2-6B	4P1-22	FQSSRVPLT	64	--	53 pM	--
20C2-7A	SJ-p2-27	LNSTTVPLT	73	128 nM	--	--
20C2-8A	SJ-p2-39	LQTTRVPLT	80	14 nM	140 pM	376 pM
20C2-8B	SJ-p2-39	LQTTRVPLT	80	--	46 pM	64 pM
20C2-9A	SJ-p2-51	VQTSLVPLT	94	36 nM	241 pM	420 pM
20C2-10A	SJ-p3-33	FQGSRLPVS	61	--	84 nM	--
20C2-11A	SJ-p3-6	FQGSLLPLS	98	--	--	--
20C2-12A	4P1-32	LQSSLVPLT	76	617 nM	1.5 nM	--
20C2-13A	4p1-20	LQTSNVPLT	79	94 nM	3 nM	--
20C2-18A	SJ-p1-9M	MQSSFVPLT	86	126 nM	1.8 nM	--
20C2-20A	SJ-p3-15	FQGSLFPPV	58		21 nM	
20C2-22A	SJ-p2-66	SQASRVPLT	90		2.3 nM	
20C2-23A	4P1-40	HQSSKVPLT	67		649 pM	1.5 nM
20C2-24A	SJ-p2-44	GQTTLVPLT	65		1.9 nM	
20C2A		FQGSLVPLT	39		27 nM	
20C2B		FQGSLVPLT	39		5.4 nM	
Mouse-20C2		FQGSLVPLT	39	83 nM	3.4 nM	

TABLE 7

Name	Clone	LC-CDR3	SEQ ID NO:	K_D (bADDL)		
				Fab	IgG1#1	IgG1#2
20C2-1A	SJ-p2-60	FQASRVPLT	52	85 nM	75 pM	--
20C2-2A	SJ-p1-18	FQATRVPLT	53	28 nM	15 pM	0.3 pM
20C2-3A	SJ-p3-16	FQGSFIPGT	55	--	3.7 nM	--
20C2-5A	SJ-p2-1F	FQSSFVPLT	63	41 nM	317 pM	68 pM
20C2-6A	4P1-22	FQSSRVPLT	64	42 nM	4.3 pM	24 pM
20C2-6B	4P1-22	FQSSRVPLT	64	--	53 pM	--
20C2-7A	SJ-p2-27	LNSTTVPLT	73	435 nM	--	--
20C2-8A	SJ-p2-39	LQTTRVPLT	80	13 nM	3 pM	0.7 pM
20C2-8B	SJ-p2-39	LQTTRVPLT	80	--	13 pM	0.8 pM
20C2-9A	SJ-p2-51	VQTSLVPLT	94	40 nM	--	2 pM
20C2-10A	SJ-p3-33	FQGSRLPVS	61	--	7.7 nM	
20C2-11A	SJ-p3-6	FQGSLLPLS	98	--	--	--
20C2-12A	4P1-32	LQSSLVPLT	76	238 nM	15 pM	--
20C2-13A	4p1-20	LQTSNVPLT	79	567 nM	764 pM	
20C2-18A	SJ-p1-9M	MQSSFVPLT	86	85 nM	149 pM	
20C2-20A	SJ-p3-15	FQGSLFPPV	58		6.9 nM	
20C2-22A	SJ-p2-66	SQASRVPLT	90		198 pM	
20C2-23A	4P1-40	HQSSKVPLT	67		85 pM	66 pM
20C2-24A	SJ-p2-44	GQTTLVPLT	65		114 pM	
20C2A		FQGSLVPLT	39			
20C2B		FQGSLVPLT	39			
Mouse-20C2		FQGSLVPLT	39	62 nM	4.1 nM	

Heavy Chain Maturation. The heavy chain of Hu20C2 was also subjected to optimization by generation of 3 libraries covering the heavy chain-CDR3 (RQLGLRSIDAMDY; SEQ ID NO:99). These libraries were designated 20C2B-39HC_3-1, 20C2B-39HC_3-2, and 20C2B-39HC_3-3 representing heavy chain CDR3 sequences of XXXXXRSIDAMDY (SEQ ID NO:100) and RQLGLRSIXXXXX (SEQ ID NO:101) and RQLGXXXXXAMDY (SEQ ID NO:102), respectively. Biotinylated reverse primers, 20C2HC3-1 (SEQ ID NO:130), 20C2HC3-2 (SEQ ID NO:133), and 20C2HC3-3 (SEQ ID NO:136) were used in combination with forward primer 20C2HC3F (SEQ ID NO:127) to generate the 3 libraries (see Figure 5B). The libraries contained $>10^8$ functional diversity and covered all combinations of amino acids at every position randomized in each set (see Table 8).

TABLE 8

Monoclonal Antibody	Diversity	Sequence	SEQ ID NO:
Hu20C2		RQLGLRSIDAMDY	99
20C2B-39HC_3-1	5.78 x 10^8	XXXXXRSIDAMDY	100
20C2B-39HC_3-2	6.16 x 10^8	RQLGLRSIXXXXX	101
20C2B-39HC_3-3	3.99 x 10^8	RQLGXXXXXAMDY	102

A total of 18 output plates from 4 rounds of panning were tested in a phage ELISA assay. A total of 1235 hits were found, of which 704 were sequenced. Based on BIACORE™ K_D values of Fab fragments against biotinylated-Aβ1-20 and biotinylated ADDL (bADDL) antigens, as well as single-point BIACORE™3000 analysis (Table 9), a total of 6 Fab clones were converted into IgG1 and IgG2m4 using either CDR grafting or veneering humanization techniques. One of these 6 Fabs, designated 4a-A3, was isolated from library 20C2B-39HC_3-3 and carried 3 amino acid substitutions (RQLGTRGTDAMDY; SEQ ID NO:3) in the middle section of the heavy chain. As is evident from Figure 2B, this heavy chain CDR3 sequence is that of Hu20C2A3.

TABLE 9

Clone	bADDL Binding K_D (M)	Aβ 1-20 Binding K_D (M)	BIACORE™ 3000 Off-rate	Sequence	SEQ ID NO:
4a-A3	8.23E-11	8.49E-10	6.65E-05	RQLGTRGTDAMDY	3
4b-A7	3.76E-10	1.72E-09	1.23E-04	RQLGKLALDAMDY	142
4b-H11	1.05E-09	1.33E-09	1.32E-04	RQLGRRSVDAMDY	8
20C28B	1.10E-09	1.10E-09	8.76E-05	RQLGLRSIDAMDY	143
4a-F5	1.39E-09	1.33E-09	1.17E-04	RQLGKLKTDAMDY	7
4a-B2	1.92E-09	1.29E-09	1.13E-04	RQLGARKTDAMDY	5
4b-D8	2.23E-09	1.69E-09	1.45E-04	RALSPRSIDAMDY	4
4a-A4	2.67E-09	1.58E-09	1.20E-04	RALSPRSIDAMDY	4
4b-A1	2.87E-09	2.85E-09	1.23E-04	RQLGPRKRDAMDY	6
4a-A7	3.24E-09	2.21E-09	1.55E-04	RQLGQRQTDAMDY	144
4a-B9	3.44E-09	3.54E-09	1.94E-04	RAIQPRSIDAMDY	145
4a-B3	4.17E-09	3.64E-09	1.59E-04	RQLGLRSIDAHTR	146
4a-G10	4.52E-09	2.72E-09	1.78E-04	RQLGQPSVDAMDY	147
4a-E11	4.93E-09	3.48E-09	1.65E-04	RQLGFQSTDAMDY	148

4a-C9	8.43E-09	2.46E-09	1.75E-04	RQLGQAGHDAMDY	149
4a-D5	1.17E-09	3.91E-09	1.74E-04	RQLGDNVADAMDY	150
4a-E10	1.85E-08	3.60E-09	1.39E-04	RQLGFQSTDAMDY	148
4b-D4	1.86E-08	4.87E-09	1.89E-04	RQLGMATPDAMDY	151
4b-B10	6.28E-08	7.43E-09	1.81E-04	RQLGAHWLDAMDY	152
4b-A12	1.54E-07	1.01E-08	1.69E-04	RQLGPEPQDAMDY	153

Example 5: Generation of IgG2m4 Antibodies

IgG2m4 antibody derivatives were prepared to decrease Fc receptor engagement, C1q binding, unwanted cytotoxicity or immunocomplex formation while maintaining both the long half-life and pharmacokinetic properties of a typical human antibody. The basic antibody format of IgG2m4 is that of IgG2, which has been shown to possess a superior half-life in experimental models (Zuckier, et al. (1994) *Cancer Suppl.* 73:794-799). The structure of IgG2 was modified to eliminate C1q binding, through selective incorporation of IgG4 sequences, while maintaining the typical low level of FcγR binding (Canfield and Morrison (1991) *J. Exp. Med.* 173:1483-1491). This was achieved by using cross-over points wherein sequences of IgG2 and IgG4 were identical, thereby producing an antibody containing natural Fc sequences rather than any artificial mutational sequences. The advantages of using the instant IgG2m4 antibody which exhibits minimal effector-related activity is comparable to the deglycosylated antibody disclosed by Wilcock et al. ((2006) *J. Neurosci.* 26:5340-6).

The IgG2m4 form of the human antibody constant region was formed by selective incorporation of human IgG4 sequences into a standard human IgG2 constant region, as shown in Figure 6. Conceptually, IgG2m4 resulted from a pair of chain-swaps within the CH2 domain as shown in Figure 6. Four single mutations were made corresponding to sequences from IgG4. The Fc residues mutated in IgG2 included His268Gln, Val309Leu, Ala330Ser, and Pro331Ser,

which minimized the potential for neoepitopes. The specific IgG4 amino acid residues placed into the IgG2 constant region are shown in Table 10, along with other alternatives from the basic structure.

TABLE 10

Residue (Kabat numbering)	Residue in IgG2	Residue in IgG4	Residue in IgG2m4	Alternative residue in IgG2m4	Comment
189	Pro or Thr*	Pro	Thr	Pro	Key polymorphism of IgG2; Pro residue present in *IGHG*01* allotype and Thr residue present in *IGHG2*02* allotype[a,b].
268	His	Gln	Gln	--	Change in the B/C loop known to be involved in FcγRII binding[c].
309	Val	Leu or Val	Leu	Val	FcRn binding domain
330	Ala	Ser	Ser	--	Key residue for C1q binding[d]; also potentially involved in binding FcγRII and FcγRIII[e].
331	Pro	Ser	Ser	--	Key residue for C1q binding[d,f] and FcγRI binding[g]; also potentially involved in binding FcγRII and FcγRIII[e].
397	Met or Val*	Val	Met	Val	Val residue present in *IGHG*01* allotype and Met residue present in *IGHG2*02* allotype[a].

*Positions marked with an asterisk are subject to allelic variations.

[a]Hougs, et al. (2001) *Immunogenetics* 52(3-4):242-8.

[b]WO 97/11971.

[c]Medgyesi, et al. (2004) *Eur. J. Immunol.* 34:1127-1135.

[d]Tao, et al. (1991) *J. Exp. Med.* 173:1025-1028.

[e]Armour, et al. (1999) *Eur. J. Immunol.* 29:2613.

[f]Xu, et al. (1994) *J. Biol. Chem.* 269:3469-3474.

[g]Canfield and Morrison (1991) *J. Exp. Med.* 173:1483.

Human IgG1/kappa and IgG2m4/kappa versions of humanized Hu20C2 and Hu20C2A3 antibodies were constructed. The complete amino acid sequence of the light and heavy

chain Hu20C2A3 IgG2m4 antibody is shown in Figures 7A and 7C.

Example 6: Binding Affinity and Specificity of Humanized Anti-ADDL Antibodies

Affinity maturation was carried out to improve affinity and improve preferential binding to ADDL. To evaluate ADDL binding affinity of the humanized antibodies, BIACORE™ and titration ELISAs were conducted as disclosed herein. Briefly, Streptavidin-coated, 96-well microtiter plates (Sigma, St. Louis, MO) were coated with 10% biotinylated ADDL antigen (1 μM). A series of 2-fold dilutions of purified antibody, starting at 500 ng/mL was added to the ADDL captured plates and the plates were incubated for 2 hours at 25°C. After washing five times with PBS solution using a plate washer (Bio-Tek, Winooski, VA), polyclonal goat anti-human kappa light chain antibody (Biomeda, Foster City, CA) was added at a 1/2000 dilution in 3% non-fat milk blocker and incubated at room temperature for 1 hour. A rabbit anti-goat IgG (H+L) HRP-conjugated (Bethyl Laboratories, Inc., Montgomery, TX) detection antibody was then added at a 1/2000 dilution in blocking solution and incubated for 1 hour at room temperature. After washing with PBS, HRP substrate, 3,3',5'5-tetramethylbenzidine (ready-to-use TMB; Sigma, St. Louis, MO) was added and the reaction was stopped after 10 minutes with 0.5 N H_2SO_4. Absorbance at wavelength of 450 nm was read in a plate reader (model VICTOR V; Perkin Elmer, Boston, MA) and data were processed using EXCEL® work sheet. Assay variations between plates were estimated within 20%.

The K_D of Fab clone A3, as measured by BIACORE™, was 849 pM against biotinylated-Aβ1-20. The K_D of the same Fab

clone was 82 pM against bADDLs, indicating that the A3 Fab demonstrated preferential binding to ADDLs. When the clone was humanized by veneering and converted to a full IgG1 molecule or full IgG2m4 molecule (*i.e.*, Hu20C2A3), the K_D values against Aβ1-20 and ADDL were below the reliable detection limit of the BIACORE™ instrument, indicating a significant improvement of Hu20C2A3's binding equilibrium constant against Aβ1-20 and ADDL as compared to Hu20C2.

Hu20C2A3, which is the veneered version of clone A3 with an IgG2m4 isotype, was expressed in both CHO and *Pichia*. The two sources of Hu20C2A3 were evaluated for their ability to interact with Aβ monomer and ADDLs by ELISA. As shown in Figure 8, Hu20C2A3 produced in either CHO (Figure 8A) or *Pichia* (Figure 8B) showed preferential ADDL binding versus Aβ40 monomer binding (6-fold). Binding constants ($IgGk_{50}$ values) as determined from these curves yielded values of 64 pM and 376 pM for ADDLs and Aβ monomer, respectively for Hu20C2A3 produced in *Pichia* and 58 pM and 361 pM for ADDLs and Aβ monomer, respectively for Hu20C2A3 produced in CHO cells.

Example 7: Inhibition of ADDL Binding to Neurons Using Humanized Anti-ADDL Antibodies

The humanized anti-ADDL antibodies were further evaluated for their ability to block ADDL binding to primary hippocampal neurons using the methods disclosed herein. Hu20C2A3 antibody, or PBS as a control, was mixed at various molar ratios with bADDLs and incubated for one hour at 37°C on a slow rotator. After the preincubation, the antibody/bADDL preparations were added to primary neuron cultures and incubated for an additional hour at 37°C. At the end of the incubation period, the bADDLs/antibody mixture was removed and the plates washed

six times with media. The cells were then fixed in 4% paraformaldehyde for ten minutes at room temperature, the solution removed, fresh fixative added, and the cells fixed for an additional ten minutes. The cells were permeabilized with 4% paraformaldehyde containing 0.1% TRITON™ X-100 (2 times, each for ten minutes at room temperature), washed six times in PBS and then treated with 10% BSA in PBS for one hour at 37°C. Alkaline phosphatase-conjugated streptavidin (1:1,500 in 1% BSA; Molecular Probes, Eugene, OR) was then added to the cells for one hour at room temperature. The cells were rinsed six times with PBS, the alkaline phosphatase substrate (CDP-STAR® with SAPPHIRE-II™; Applied Biosystems, Foster City, CA) added to the cells and incubated for thirty minutes prior to determining the luminescence on a LJL Luminometer (Analyst AD; LJL Biosystems, Sunnyvale, CA). In this analysis, Hu20C2A3 was found to effectively inhibit bADDL binding to neurons at a sub-stoichiometric antibody to peptide ratio (EC_{50} = 0.16; Figure 9).

The inhibition of bADDL binding indicated that Hu20C2A3 interacts with ADDLs in a biologically relevant manner. To demonstrate that Hu20C2A3 interacts with ADDLs relevant to the human Alzheimer's Disease condition, the ability of biotinylated Hu20C2A3 to immuno-label Aβ containing plaques in human Alzheimer's Disease brain tissue was evaluated. Immunohistochemical localization showed avid labeling of Aβ in both dense core and diffuse plaques in human Alzheimer's Disease brain tissue. The specificity of this binding was demonstrated by a loss of immunoreactivity following pre-incubation with increasing ADDL:antibody amounts. Similar to Hu20C2, Hu20C2A3, efficiently labels both dense core and diffuse Aβ deposits.

Example 8: Thermal Stability of Hu20C2A3

An evaluation of the protein stability of Hu20C2A3 was assessed using SEC-HPLC, fluorescent thermal melt analysis, and particle size analysis. Fluorescent thermal melt analysis indicated that Fc and Fab unfolding transitions occurred at approximately 70°C and 80°C, respectively, consistent with acceptable inherent protein stability (Figure 10).

Example 9: *In Vivo* Pharmacodynamic and Efficacy Analysis

The prior art indicates that systemic injection of monoclonal anti-Aβ antibodies can increase plasma levels of Aβ acutely, whereas measurable lowering of brain Aβ requires chronic administration. It has been suggested that passive immunization in species with measurable Aβ results in an elevation of plasma Aβ due to a change in the equilibrium of Aβ between brain and peripheral compartments. This "peripheral sink" ultimately leads to lowering of brain Aβ. However, cognitive improvement has been observed in animals following acute antibody administration prior to notable changes in brain Aβ, indicating that changes in brain Aβ may occur in some form prior to a time point where these changes can be measured using known techniques. Alternately, elevations of plasma Aβ could be explained by a stabilization of peripheral Aβ following administration of antibody. Regardless of the interpretation, it has been established that early plasma elevations are a prerequisite for subsequent lowering of brain Aβ in animal models. Thus, the effect of Hu20C2A3 antibodies on plasma Aβ elevations were utilized as an indicator of target engagement. Following infusion of Hu20C2A3 at doses of 30, 100 and 300 µg/mouse IV, significant and robust increases in plasma Aβx-40 were

observed relative to the non-relevant antibody (8B4) control group 4 hours post-injection (Figure 11). The observed increases in plasma Aβx-40 for CHO-derived material were 491% (30 μg, $p>0.001$), 826% (100 μg, $p<0.001$), and 755% (300 μg, $p<0.001$) of the 8B4 levels. Similarly, the increases in plasma Aβx-40 for *Pichia*-derived material were 395% (30 μg, $p>0.001$), 729% (100 μg, $p<0.001$), and 838% (300 μg, $p<0.001$) of the 8B4 levels.

What is claimed is:

1. An isolated antibody, or fragment thereof, capable of differentially recognizing a multi-dimensional conformation of one or more Aβ-derived diffusible ligands, wherein said antibody comprises a complementary determining region (CDR) of Arg-Xaa_1-Leu-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa_1 is Gln or Ala; Xaa_2 is Ser or Gly; Xaa_3 is Pro, Ala, Lys, Arg, or Thr; Xaa_4 is Lys or Arg; Xaa_5 is Gly, Ser, or Lys; Xaa_6 is Val, Thr, Ile or Arg.

2. The isolated antibody of claim 1, wherein the heavy and light chain variable regions of said antibody respectively comprise the amino acid sequence set forth in SEQ ID NO:108 and SEQ ID NO:112.

3. The isolated antibody of claim 1, wherein the heavy and light chain of said antibody respectively comprise the amino acid sequence set forth in SEQ ID NO:138 and SEQ ID NO:140.

4. A pharmaceutical composition comprising an isolated antibody of claim 1, or fragment thereof, capable of differentially recognizing a multi-dimensional conformation of one or more Aβ-derived diffusible ligands in admixture with a pharmaceutically acceptable carrier.

5. A method for preventing binding of Aβ-derived diffusible ligands to a neuron comprising contacting the neuron with the antibody of claim 1 so that binding of Aβ-derived diffusible ligands to the neuron is prevented.

6. A method for inhibiting assembly of Aβ-derived diffusible ligands comprising contacting a sample containing amyloid β 1-42 peptides with the antibody of claim 1 thereby inhibiting assembly of Aβ-derived diffusible ligands.

7. A method for blocking the phosphorylation of tau protein at Ser202/Thr205 comprising contacting a sample containing a tau protein with the antibody of claim 1 thereby blocking the phosphorylation of tau protein at Ser202/Thr205.

8. A method for prophylactically or therapeutically treating a disease associated with Aβ-derived diffusible ligands comprising administering an effective amount of the pharmaceutical composition of claim 4.

9. A method for identifying a therapeutic agent that prevents the binding of Aβ-derived diffusible ligands to a neuron comprising contacting a neuron with Aβ-derived diffusible ligands in the presence of an agent and using an antibody of claim 1 to determine binding of Aβ-derived diffusible ligands to the neuron in the presence of the agent.

10. A method for detecting Aβ-derived diffusible ligands in a sample comprising contacting a sample with the antibody of claim 1 so that Aβ-derived diffusible ligands are detected.

11. A method for diagnosing a disease associated with Aβ-derived diffusible ligands comprising contacting a

sample with the antibody of claim 1 so that a disease associated with Aβ-derived diffusible ligands is diagnosed.

12. A kit for detecting Aβ-derived diffusible ligands comprising the isolated antibody, or fragment thereof, of claim 1.

20C2 - HEAVY CHAIN VARIABLE REGION SEQUENCE
tgggcagacttaccattctcattcctgctgctgattgtccctgcatatg
tcttgtccCAAGTTACTCTAAAAGAGTCTGGCCCTGGGATATTGAAGCC
CTCACAGACCCTCAGTCTGACTTGTTCTCTCTCTGGGTTTTCACTGAGC
ACTTCTGGTATGGGTGTAGGCTGGTTTCGTCAGCCTTCAGGGAAGGGTC
TGGAGTGGCTGGCACACATTTGGTGGGATGATGATAAGTCCTATAATCC
ATCCCTGAAGAGCCGGCTCACAATCTCCAAGTATACCTCTAGAAACCAG
GTTTTCCTCACGATCACCAGTGTGGACACTGCAGATACTGCCACTTACT
ATTGTGCTCGAAGACAACTCGGACTAAGATCAATTGATGCTATGGACTA
CTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACACCC
CCATCTGTCTATCCACTG (SEQ ID NO:103)

FIG. 1A

20C2 - LIGHT CHAIN VARIABLE REGION SEQUENCE
agattgcctgttaggctgttggtgctgatgttctggattcctgcttcca
ccagtGATGTTTTGATGACCCAAACTCCTCTCTCCCTGCCTGTCAGTCT
TGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTCTACAT
AGTAATGGAAACACCTATTTAGAGTGGTACCTGCAGAAACCAGGCCAGT
CTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCC
AGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATC
AGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGTTTTCAAGGTT
CACTTGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACG
GGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGT
(SEQ ID NO:104)

FIG. 1B

```
                20C2 QVTLKESGPG ILKPSQTLSL TCSLSGFSLS TSGMGVGWFR QPSGKGLEWL
      VH2 3-1 2-70 QVTLKESGPA LVKPTQTLTL TCTFSGFSLS TSGMRVSWIR QPPGKALEWL
            Hu20C  QVTLKESGPA LVKPTQTLTL TCTLSGFSLS TSGMGVGWIR QPPGKALEWL
      Hu20C2A3 QVTLKESGPG LLKPTQTLTL TCTLSGFSLS TSGMGVGWFR QPPGKGLEWL
                                          HCDR1

                20C2 AHIWWDDDKS YNPSLKSRLT ISKYTSRNQV FLTITSVDTA DTATYYCARR
      VH2 3-1_2-70 ARIDWDDDKF YSTSLKTRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARI
            Hu20C2 AHIWWDDDKS YNPSLKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARR
      Hu20C2A3 AHIWWDDDKS YNPSLKSRLT ISKDTSKNQV VLTITNVDPV DTATYYCARR
                                HCDR2

                      * **
                20C2 QLGLRSIDAMDYW GQGTSVTVSS (SEQ ID NO:105)
               (JH6)   YYYYYGMDVW GQGTTVTVSS (SEQ ID NO:106)
            Hu20C2 QLGLRSIDAMDYW GQGTTVTVSS (SEQ ID NO:107)
      Hu20C2A3 QLGTRGTDAMDYW GQGTTVTVSS (SEQ ID NO:108)
                      HCDR3
```

FIG. 2A

20C2 DV**L**MTQTPLS LPV**SL**G**DQ**AS ISCRSSQSIL HSNGNTYLEW YLQKPGQSP**K**
VKII 4-1-(1)A18 DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDGKTYLYW YLQKPGQSPQ
Hu20C2 DVVMTQSPLS LPVTPGEPAS ISCRSSQSIL HSNGNTYLEW YLQKPGQSPQ
Hu20C2A3 DV**V**MTQTPLS LPV**TP**G**QP**AS ISCRSSQSIL HSNGNTYLEW YLQKPGQSP**Q**
LCDR1

* ***
20C2 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAED**L**GV YYC**F**Q**GSL**VP
VKII_4-1-(1)A18 LLIYEVSSRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCMQGIHLP
Hu20C2 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCLQTTRVP
Hu20C2A3 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAED**V**GV YYC**L**Q**TTR**VP
LCDR2 LCDR3

101
20C2 LTFG**A**GTKLE **L**K (SEQ ID NO:109)
(JK2) YTFGQGTKLE IK (SEQ ID NO:110)
Hu20C2 LTFGQGTKLE IK (SEQ ID NO:111)
Hu20C2A3 LTFG**Q**GTKLE **I**K (SEQ ID NO:112)

FIG. 2B

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5332

HUMANIZED Hu20C2 (CDR GRAFTED) - HCVRA
caggtgaccctgaaggagtctggccctgccctggtgaagcccacccaga
ccctgaccctgacctgcaccttctctggcttcagcctgagcacctctgg
catgggcgtgggctggatccggcagccccctggcaaggccctggagtgg
ctggcccacatctggtgggacgacgacaagtcctacaaccccagcctga
agagccggctgaccatcagcaaggacaccagcaagaaccaggtggtgct
gaccatgaccaacatggaccctgtggacacagccacctactactgtgcc
cggcggcagctgggcctgcggagcattgatgccatggactactggggcc
agggcaccacagtgacagtgtccagc (SEQ ID NO:113)

FIG. 3A

HUMANIZED Hu20C2 (CDR GRAFTED) - HCVRB
caggtgaccctgaaggagtctggccctgccctggtgaagcccacccaga
ccctgaccctgacctgcaccctgtctggcttcagcctgagcacctctgg
catgggcgtgggctggatccggcagccccctggcaaggccctggagtgg
ctggcccacatctggtgggacgacgacaagtcctacaaccccagcctga
agagccggctgaccatcagcaaggacaccagcaagaaccaggtggtgct
gaccatgaccaacatggaccctgtggacacagccacctactactgtgcc
cggcggcagctgggcctgcggagcattgatgccatggactactggggcc
agggcaccacagtgacagtgtccagc (SEQ ID NO:114)

FIG. 3B

HUMANIZED Hu20C2 (CDR GRAFTED) - LCVR
gatgtggtgatgacccagagcccccctgtccctgcctgtgaccctggcg
agcctgccagcatctcctgccggagctcccagagcatcctgcactccaa
tggcaacacctacctggagtggtacctgcagaagcctggccagagcccc
cagctgctgatctacaaggtgtccaaccggttctccggcgtgcctgacc
ggttcagcggctccggcagcggcacagacttcaccctgaagatcagccg
ggtggaggctgaggatgtgggcgtctactactgcttccagggcagcctg
gtgcccctgacctttggccagggcaccaagctggagatcaag (SEQ
ID NO:115)

FIG. 3C

pFab4 Hu20C2 HEAVY CHAIN VERSION A

MKKTAIAIAVALAGFATVAQAALE*QVTLKESGPALVKPTQTLTLTCTFS*
ompA secretion signal Hu20C2

GFSLSTSGMGVGWIRQPPGKALEWLAHIWWDDDKSYNPSLKSRLTISKD
CDR1 CDR2

T*SKNQVVLTMTNMDPVDTATYYCARRQLGLRSIDAMDYWGQGTTVTVSS*
CDR3

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCTSGHHHHHHGGEQKLISEEDLGG*PFVCEYQGQSSDLPQPPVNA
hCH1 HIS-TAG MYC-TAG ♦Amb

GGGSGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSGSGDFDYEKMANA
pIII stump

NKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVSGLANGNGA
TGDFAGSNSQMAQVGDGDNSPLMNNFRQYLPSLPQSVECRPYVFGAGKP
YEFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES* (SEQ
ID NO:116)

FIG. 4A

pFab4 Hu20C2 HEAVY CHAIN VERSION B

MKKTAIAIAVALAGFATVAQAALEQVTLKESGPALVKPTQTLTLTCTLS
ompA secretion signal Hu20C2

GFSLSTSGMGVGWIRQPPGKALEWLAHIWWDDDKSYNPSLKSRLTISKD
CDR1 CDR2

TSKNQVVLTMTNMDPVDTATYYCARRQLGLRSIDAMDYWGQGTTVTVSA
CDR3

STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE
PKSCTSGHHHHHHGGEQKLISEEDLGG*PFVCEYQGQSSDLPQPPVNAG
hCH1 HIS-TAG MYC-TAG ♦Amb

GGSGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSGSGDFDYEKMANAN
pIII stump

KGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVSGLANGNGAT
GDFAGSNSQMAQVGDGDNSPLMNNFRQYLPSLPQSVECRPYVFGAGKPY
EFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES* (SEQ
ID NO:117)

FIG. 4B

pFab4 Hu20C2 LIGHT CHAIN

MKYLLPTAAAGLLLLAAQPAMASRDVVMTQSPLSLPVTPGEPASISCRS
Pel secretion signal Hu20C2

SQSILHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGT
CDR1 CDR2

DFTLKISRVEAEDVGVYYCFQGSLVPLTFGQGTKLEIKRTVAAPSVFIF
CDR3

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
hCK (SEQ ID NO:118)

FIG. 4C

pFab4 Hu20C2 HEAVY CHAIN VERSION A/LIGHT CHAIN

GCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGC
ACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTT
AGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT
GTGGAATTGTGAGCGGATAACAATTTCACACAAGATCTAGCTATTCCAGAGATTACG
CAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTGCTGCCGACTGCAGCTGC
TGGTCTGCTGCTGCTGGCGGCCCAGCCGGCTATGGCTTCTAGAgatgtggtgatgac
ccagagccccctgtccctgcctgtgaccccctggcgagcctgccagcatctcctgccg
gagctcccagagcatcctgcactccaatggcaacacctacctggagtggtacctgca
gaagcctggccagagcccccagctgctgatctacaaggtgtccaaccggttctccgg
cgtgcctgaccggttcagcggctccggcagcggcacagacttcaccctgaagatcag
ccgggtggaggctgaggatgtgggcgtctactactgcttccagggcagcctggtgcc
cctgacctttggccagggcaccaagctggagatcaagCGTACGGTGGCTGCACCATC
TGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGT
GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAA
CGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAG
CACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAA
AGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTT
CAACAGGGGAGAGTGTTAACAATTGCTAGAATTGTGAGCGGATAACAATTTCAGCAG
GTCGAGTTCTTGATAACGAGGCGTAAAAAATGAAAAAGACAGCTATCGCGATTGCAG
TGGCACTGGCTGGTTTCGCTACCGTGGCCCAGGCGGCCCTCGAGcaggtgaccctga
aggagtctggccctgccctggtgaagcccacccagaccctgaccctgacctgcacct
tctctggcttcagcctgagcacctctggcatgggcgtgggctggatccggcagcccc
ctggcaaggccctggagtggctggcccacatctggtgggacgacgacaagtcctaca
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tgctgaccatgaccaacatggaccctgtggacacagccacctactactgtgcccggc
ggcagctgggcctgcggagcattgatgccatggactactggggccagggcaccacag
tgacagtgtccagcGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCT
CCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCT
TCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGC
CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTACTAGTGGCCACCACCACC
ATCACCATGGCGGTGAACAAAAACTCATCTCAGAAGAGGATCTGGGTGGTTAGCCAT
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GCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTGGCGGCTCCGGTTCCG
GTGATTTTGATTATGAAAAAATGGCAAACGCTAATAAGGGGGCTATGACCGAAAATG
CCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTG
ATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTA
ATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACG
GTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCTTTGCCTCAGT
CGGTTGAATGTCGCCCTTATGTCTTTGGCGCTGGTAAACCATATGAATTTTCTATTG
ATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCA
CCTTTATGTATGTATTTTCGACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAAG
CTAGCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACT
TAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCG
CACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGATTCCGTTG

FIG. 4D

CAATGGCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTT
CTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCGACAACGGTTA
ATTTGCGTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTT
CTCAGGATTCTGGCGTACCGTTCCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTA
GCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCA
TAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGC
GTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCC
TTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTA
GGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGAT
GGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAG
TCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATC
TCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAA
AATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACA
ATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACC
GGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGCAGGTGGCACT
TTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAAT
ATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG
AAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTT
TGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGAT
CAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT
GAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA
TGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATA
CACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACG
GATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACT
GCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTG
CACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
GCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTG
CGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGAC
TGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGC
TGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGT
CAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATT
AAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAA
CTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACC
AAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATC
AAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAA
AAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTT
CCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTG
CTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCG
TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGT
GAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTA
AGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGG
TATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGAT
TCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGA
ACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGC (SEQ ID
NO:119)

FIG. 4E

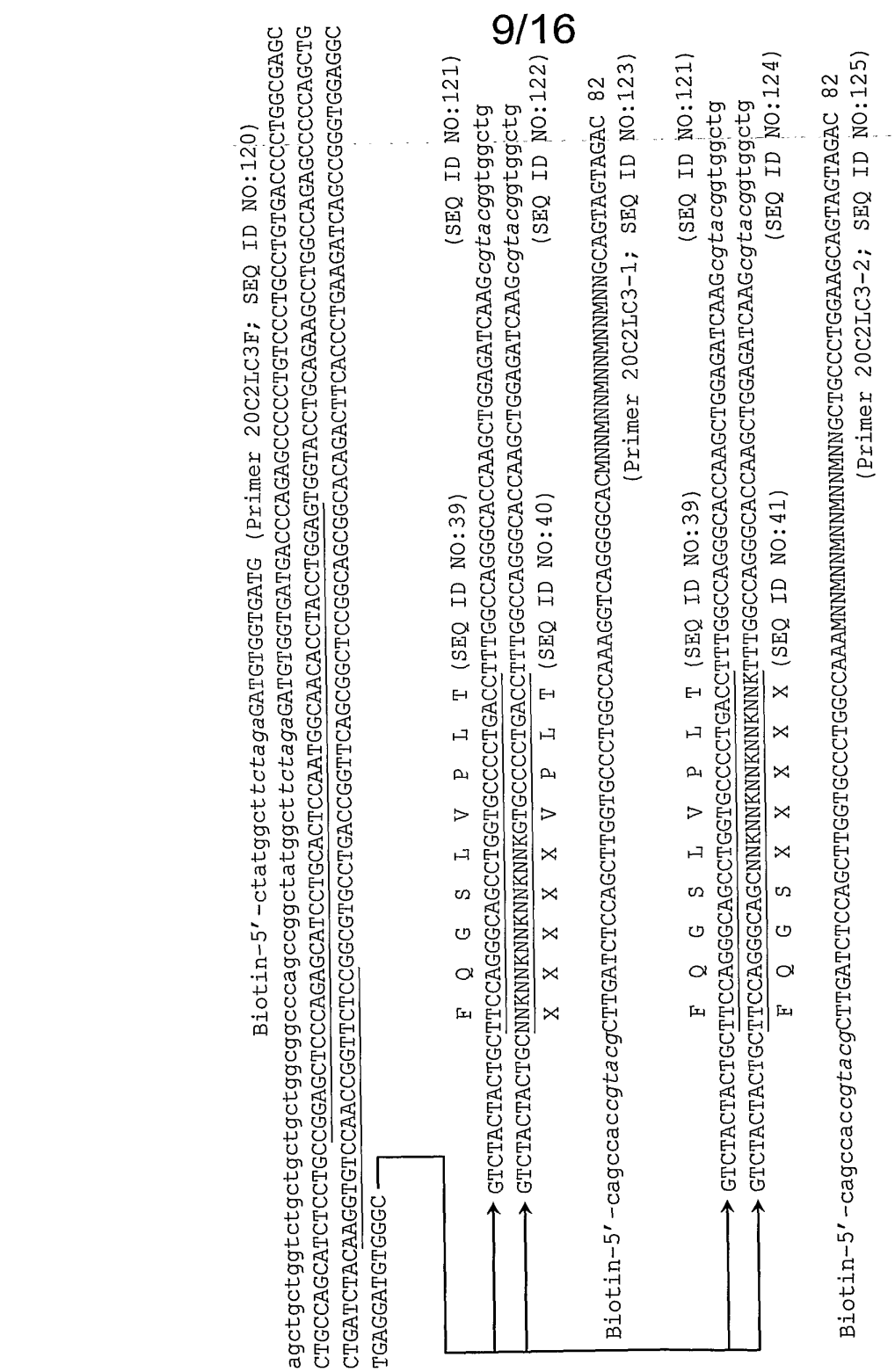

Biotin-5′-ctatggcttctagaGATGTGGTGATG (Primer 20C2LC3F; SEQ ID NO:120)
agctgctggtctgctgctgctggcggcccagccggctatggcttctagaGATGTGGTGATGACCCAGAGCCCCCTGTCCCTGCCTGTGACCCCTGGCGAGC
CTGCCAGCATCTCCTGCCGGAGCTCCCAGAGCATCCTGCACTCCAATGGCAACACCTACCTGGAGTGGTACCTGCAGAAGCCTGGCCAGAGCCCCCAGCTG
CTGATCTACAAGGTGTCCAACCGGTTCTCCGGCGTGCCTGACCGGTTCAGCGGCTCCGGCAGCGGCACAGACTTCACCCTGAAGATCAGCCGGGTGGAGGC
TGAGGATGTGGGC
F Q G S L V P L T (SEQ ID NO:39) (SEQ ID NO:121)
GTCTACTACTGCTTCCAGGGCAGCCTGGTGCCCCTGACCTTTGGCCAGGGCACCAAGCTGGAGATCAAGcgtacggtggctg
GTCTACTACTGCNNKNNKNNKNNKGTGCCCCTGACCTTTGGCCAGGGCACCAAGCTGGAGATCAAGcgtacggtggctg
X X X X X V P L T (SEQ ID NO:40) (SEQ ID NO:122)
Biotin-5′-cagccaccgtacgCTTGATCTCCAGCTTGGTGCCCTGGCCAAAGGTCAGGGGCACMNNMNNMNNMNNMNNGCAGTAGTAGAC 82
(Primer 20C2LC3-1; SEQ ID NO:123)
F Q G S L V P L T (SEQ ID NO:39) (SEQ ID NO:121)
GTCTACTACTGCTTCCAGGGCAGCCTGGTGCCCCTGACCTTTGGCCAGGGCACCAAGCTGGAGATCAAGcgtacggtggctg
GTCTACTACTGCTTCCAGGGCAGCNNKNNKNNKNNKNNKTTTGGCCAGGGCACCAAGCTGGAGATCAAGcgtacggtggctg
F Q G S X X X X X (SEQ ID NO:41) (SEQ ID NO:124)
Biotin-5′-cagccaccgtacgCTTGATCTCCAGCTTGGTGCCCTGGCCAAAMNNMNNMNNMNNMNNGCTGCCCTGGAAGCAGTAGTAGAC 82
(Primer 20C2LC3-2; SEQ ID NO:125)

FIG. 5A

Biotin-5'-gtttatctcgagCAGGTGACCCTGAAG (Primer 20C2HC3F; SEQ ID NO:126)
ccgtggcccaggcggccctcgagCAGGTGACCCTGAAGGAGTCTGGCCCTGCCCTGGTGAAGCCCACCCAGACCCTGACCCTGCACCTTCT
CTGGCTTCAGCCTGAGCACCTCTGGCATGGGCGTGGGCTGGATCCGGCAGCCCCCTGGCAAGGGCCCTGGAGTGGCTGGCCCACATCTGGTGGGACG
ACGACAAGTCCTACAACCCCAGCCTGAAGAGCCGGCTGACCATCAGCAAGGACACCAGCAAGAACCAGGTGGTGCTGACCATGACCAACATGGACC
CTGTGGACA
R Q L G L R S I D A M D Y (SEQ ID NO:99) (SEQ ID NO:127)
GTGCCCGGCGGCAGCTGGGCCTGCGGAGCATTGATGCCATGGACTACTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg
GTGCCCGGNNKNNKNNKNNKCGGAGCATTGATGCCATGGACTACTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg (SEQ ID NO:128)
X X X X R S I D A M D Y (SEQ ID NO:100)
Biotin-5'-CCGATGGGCCCTTGGTGGAGGCGCTGGACACTGTCACTGTGGTGCCCTGGCCCCAGTAGTCCATGGCATCAATGCTCCGMNNMNNMNNMNNMNNCC
GGGCAC (Primer 20C2HC3-1; SEQ ID NO:129)
R Q L G L R S I D A M D Y (SEQ ID NO:99) (SEQ ID NO:127)
GTGCCCGGCGGCAGCTGGGCCTGCGGAGCATTGATGCCATGGACTACTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg
GTGCCCGGCGGCAGCTGGGCCTGCGGAGCATTNNKNNKNNKNNKNNKTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg (SEQ ID NO:130)
R Q L G L R S I X X X X X X (SEQ ID NO:101)
Biotin-5'-CCGATGGGCCCTTGGTGGAGGCGCTGGACACTGTCACTGTGGTGCCCTGGCCCCAMNNMNNMNNMNNMNNAATGCTCCGCAGGCCCAGCTG
(Primer 20C2HC3-2; SEQ ID NO:131)
R Q L G L R S I D A M D Y (SEQ ID NO:99) (SEQ ID NO:127)
GTGCCCGGCGGCAGCTGGGCCTGCGGAGCATTGATGCCATGGACTACTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg
GTGCCCGGCGGCAGCTGGGCNNKNNKNNKNNKNNKGCCATGGACTACTGGGGCCAGGGCACCACAGTGACAGTGTCCAGCGCCTCCACCAAgggcccatcgg (SEQ ID NO:132)
R Q L G X X X X X A M D Y (SEQ ID NO:102)
Biotin-5'-CCGATGGGCCCTTGGTGGAGGCGCTGGACACTGTCACTGTGGTGCCCTGGCCCCAGTAGTCCATGGCMNNMNNMNNMNNMNNMNNGCCCAGCTG
(Primer 20C2HC3-3; SEQ ID NO:133)

FIG. 5B

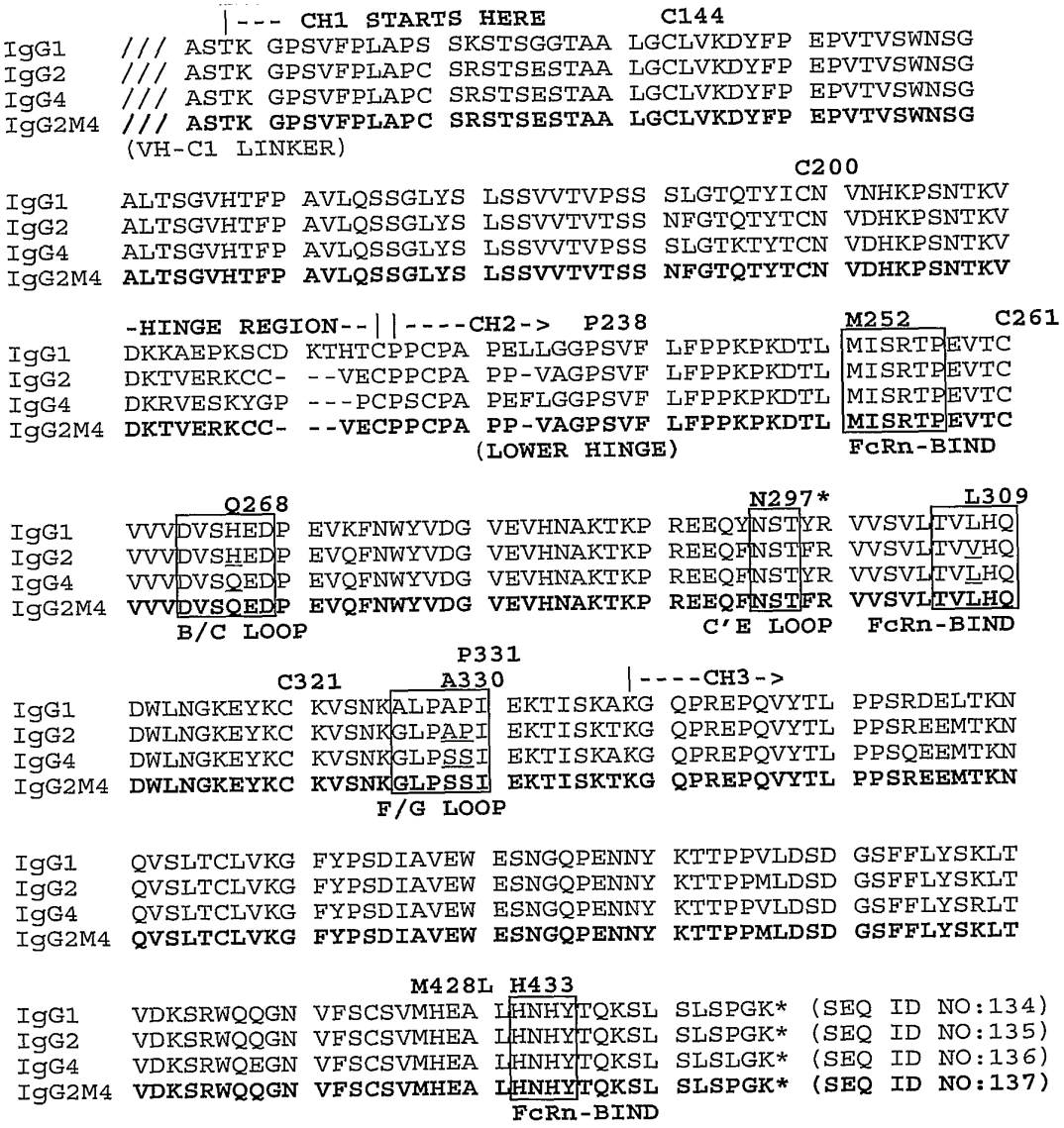

|--- CH1 STARTS HERE C144
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IgG2 /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
IgG4 /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
IgG2M4 /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
(VH-C1 LINKER)
C200
IgG1 ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV
IgG2 ALTSGVHTFP AVLQSSGLYS LSSVVTVTSS NFGTQTYTCN VDHKPSNTKV
IgG4 ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTKTYTCN VDHKPSNTKV
IgG2M4 ALTSGVHTFP AVLQSSGLYS LSSVVTVTSS NFGTQTYTCN VDHKPSNTKV
-HINGE REGION--||----CH2-> P238 M252 C261
IgG1 DKKAEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC
IgG2 DKTVERKCC- --VECPPCPA PP-VAGPSVF LFPPKPKDTL MISRTPEVTC
IgG4 DKRVESKYGP ---PCPSCPA PEFLGGPSVF LFPPKPKDTL MISRTPEVTC
IgG2M4 DKTVERKCC- --VECPPCPA PP-VAGPSVF LFPPKPKDTL MISRTPEVTC
(LOWER HINGE) FcRn-BIND
Q268 N297* L309
IgG1 VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ
IgG2 VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ
IgG4 VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR VVSVLTVLHQ
IgG2M4 VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVLHQ
B/C LOOP C'E LOOP FcRn-BIND
P331
C321 A330 |----CH3->
IgG1 DWLNGKEYKC KVSNKALPAPI EKTISKAKG QPREPQVYTL PPSRDELTKN
IgG2 DWLNGKEYKC KVSNKGLPAPI EKTISKTKG QPREPQVYTL PPSREEMTKN
IgG4 DWLNGKEYKC KVSNKGLPSSI EKTISKAKG QPREPQVYTL PPSQEEMTKN
IgG2M4 DWLNGKEYKC KVSNKGLPSSI EKTISKTKG QPREPQVYTL PPSREEMTKN
F/G LOOP
IgG1 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT
IgG2 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT
IgG4 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSRLT
IgG2M4 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT
M428L H433
IgG1 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:134)
IgG2 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:135)
IgG4 VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK* (SEQ ID NO:136)
IgG2M4 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:137)
FcRn-BIND

FIG. 6

HUMANIZED Hu20C2A3 IgG2M4 - HEAVY CHAIN

```
  1 QVTLKESGPG LLKPTQTLTL TCTLSGFSLS TSGMGVGWFR QPPGKGLEWL
 51 AHIWWDDDKS YNPSLKSRLT ISKDTSKNQV VLTITNVDPV DTATYYCARR
101 QLGTRGTDAM DYWGQGTTVT VSSASTKGPS VFPLAPCSRS TSESTAALGC
151 LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVTSSNFG
201 TQTYTCNVDH KPSNTKVDKT VERKCCVECP PCPAPPVAGP SVFLFPPKPK
251 DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS
301 TFRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK TKGQPREPQV
351 YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPML
401 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
```

(SEQ ID NO:138)

FIG. 7A

HUMANIZED Hu20C2A3 IgG2M4 - HEAVY CHAIN

```
ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACTACAGGTGT
CCACTCGCAGGTGACCCTGAAGGAGTCTGGCCCTGGCCTGCTGAAGCCCA
CCCAGACCCTGACCCTGACCTGCACCCTGTCTGGCTTCAGCCTGAGCACC
TCTGGCATGGGCGTGGGCTGGTTCCGGCAGCCCCCTGGCAAGGGCCTGGA
GTGGCTGGCCCACATCTGGTGGGACGACGACAAGTCCTACAACCCCAGCC
TGAAGAGCCGGCTGACCATCAGCAAGGACACCAGCAAGAACCAGGTGGTG
CTGACCATCACCAACGTGGACCCTGTGGACACAGCCACCTACTACTGTGC
CCGGCGGCAGCTGGGCACTAGGGGGACGGATGCCATGGACTACTGGGGCC
AGGGCACCACAGTGACAGTGTCCAGCGCATCCACCAAGGGCCCATCCGTC
TTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCT
GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGACCTCCAGCAA
CTTTGGCACGCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACA
CCAAGGTGGACAAGACAGTTGAGCGGAAATGCTGCGTGGAGTGCCCACCA
TGCCCAGCACCTCCAGTGGCCGGACCATCAGTCTTCCTGTTCCCCCCAAA
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TGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTG
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GGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCG
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ACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG
TCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCC
CATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTAACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAATGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCTGG
TAAATGA (SEQ ID NO:139)
```

FIG. 7B

HUMANIZED Hu20C2A3 - LIGHT CHAIN

1 DVVMTQTPLS LPVTPGQPAS ISCRSSQSIL HSNGNTYLEW YLQKPGQSPQ

51 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCLQTTRVP

101 LTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK

151 VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE

201 VTHQGLSSPV TKSFNRGEC (SEQ ID NO:140)

FIG. 7C

HUMANIZED Hu20C2A3 - LIGHT CHAIN

ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGA
TGCCAGATGCGATGTGGTGATGACCCAGACCCCCCTGTCCCTGCCTGTGA
CCCCTGGCCAGCCTGCCAGCATCTCCTGCCGGAGCTCCCAGAGCATCCTG
CACTCCAATGGCAACACCTACCTGGAGTGGTACCTGCAGAAGCCTGGCCA
GAGCCCCCAGCTGCTGATCTACAAGGTGTCCAACCGGTTCTCCGGCGTGC
CTGACCGGTTCAGCGGCTCCGGCAGCGGCACAGACTTCACCCTGAAGATC
AGCCGGGTGGAGGCTGAGGATGTGGGCGTCTACTACTGCCTTCAGACTAC
TCGTGTGCCCCTGACCTTTGGCCAGGGCACCAAGCTGGAGATCAAGCGTA
CGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG
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CCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA
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TCAACAGGGGAGAGTGTTAG (SEQ ID NO:141)

FIG. 7D

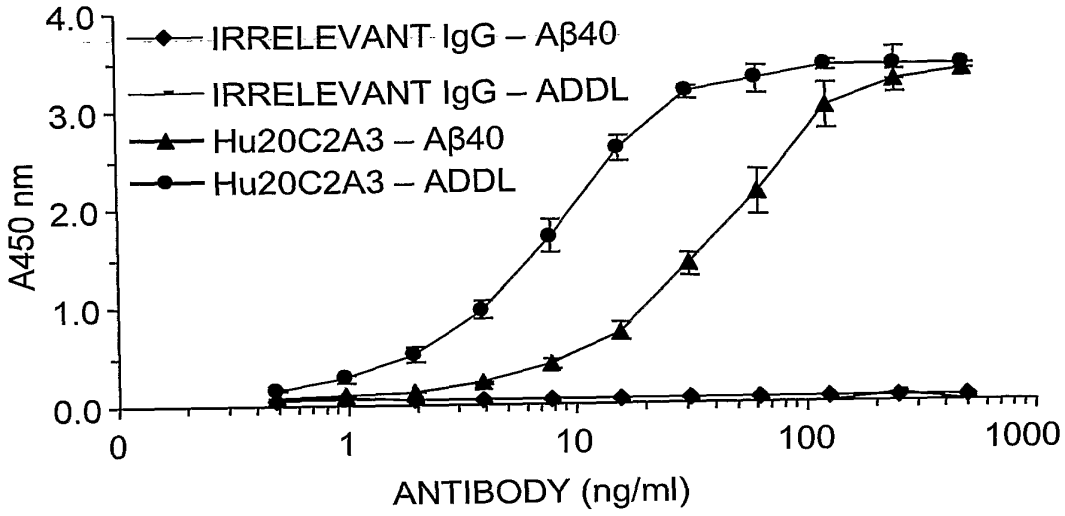

4.0
3.0
2.0
1.0
0.0
A450 nm
IRRELEVANT IgG – Aβ40
IRRELEVANT IgG – ADDL
Hu20C2A3 – Aβ40
Hu20C2A3 – ADDL
0
1
10
100
1000
ANTIBODY (ng/ml)

FIG. 8A

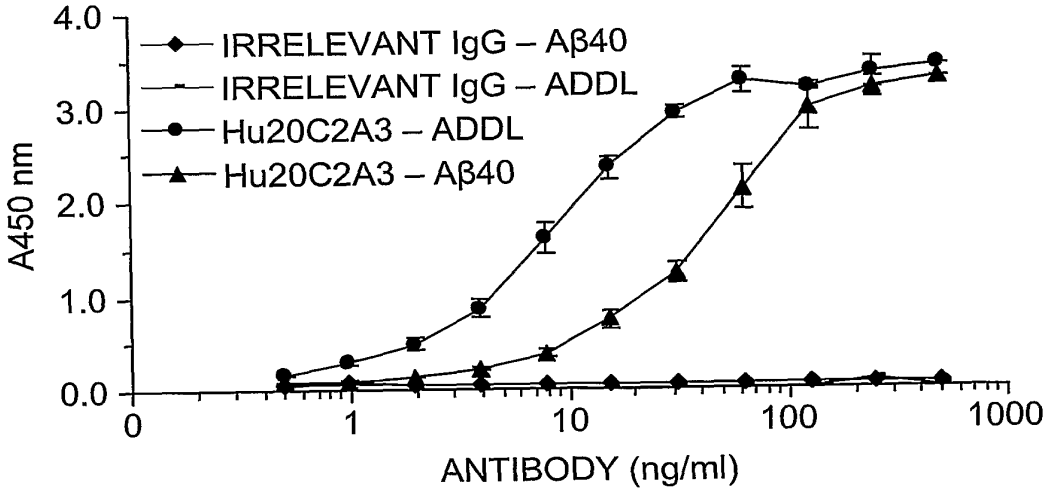

4.0
3.0
2.0
1.0
0.0
A450 nm
IRRELEVANT IgG – Aβ40
IRRELEVANT IgG – ADDL
Hu20C2A3 – ADDL
Hu20C2A3 – Aβ40
0
1
10
100
1000
ANTIBODY (ng/ml)

FIG. 8B

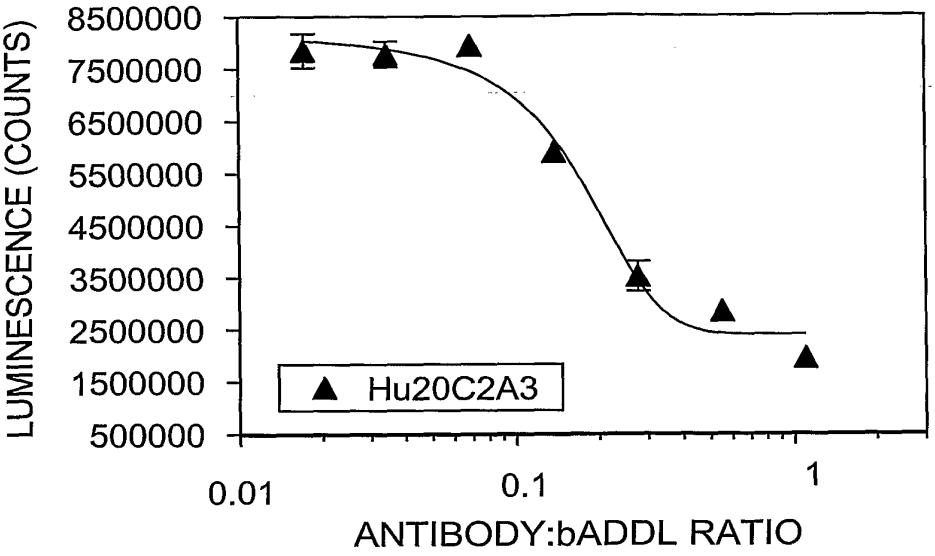

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7500000
6500000
5500000
4500000
3500000
2500000
1500000
500000
Hu20C2A3
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0.1
1
ANTIBODY:bADDL RATIO

FIG. 9

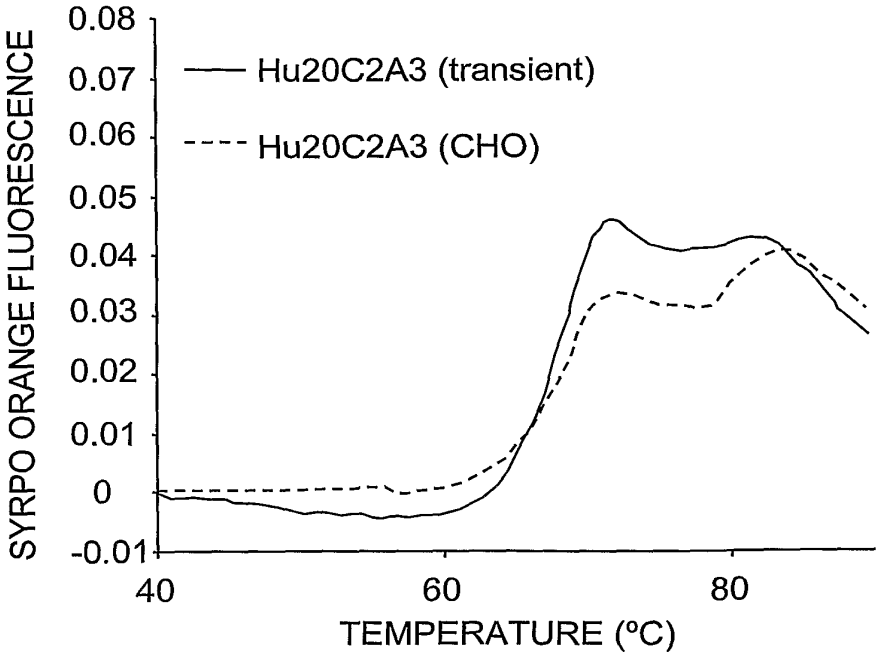

SYRPO ORANGE FLUORESCENCE
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
Hu20C2A3 (transient)
Hu20C2A3 (CHO)
40
60
80
TEMPERATURE (°C)

FIG. 10

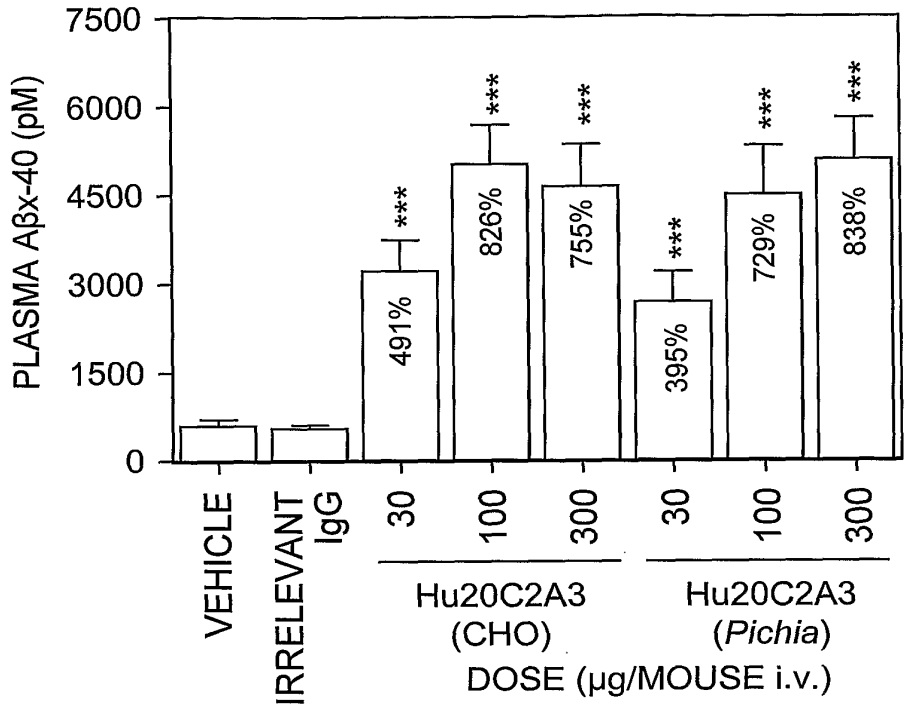

7500
6000
4500
3000
1500
0
PLASMA Aβx-40 (pM)
***
491%
826%
755%
395%
729%
838%
VEHICLE
IRRELEVANT IgG
30
100
300
30
100
300
Hu20C2A3 (CHO)
Hu20C2A3 (Pichia)
DOSE (µg/MOUSE i.v.)

FIG. 11

SEQUENCE LISTING

<110> Kinney, Gene
Strohl, William R.
An, Zhiqiang

<120> ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF

<130> MRK0003WO

<150> PCT/US2005/038125
<151> 2005-10-21

<160> 153

<170> PatentIn version 3.3

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<400> 9

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1 5

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1 5

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1 5

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Phe Gln Gly Ser Phe Ile Pro Gly Thr
1 5

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<400> 56

Phe Gln Gly Ser Phe Leu Pro Pro Ser
1 5

<210> 57
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Phe Gln Gly Ser Phe Leu Pro Gln Leu
1 5

<210> 58
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<400> 58

Phe Gln Gly Ser Leu Phe Pro Pro Val
1 5

<210> 59

<211> 9
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<213> Artificial Sequence

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<400> 59

Phe Gln Gly Ser Leu Phe Ser Pro Ser
1 5

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<400> 60

Phe Gln Gly Ser Arg Ile Pro Ile Ser
1 5

<210> 61
<211> 9
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<220>
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<400> 61

Phe Gln Gly Ser Arg Leu Pro Val Ser
1 5

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<211> 9
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<220>
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<400> 62

Phe Gln Gly Ser Arg Val Pro Leu Val
1 5

<210> 63
<211> 9
<212> PRT
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<220>
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<400> 63

Phe Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 64
<211> 9
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<220>
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<400> 64

Phe Gln Ser Ser Arg Val Pro Leu Thr
1 5

<210> 65
<211> 9
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<213> Artificial Sequence

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<400> 65

Gly Gln Thr Thr Leu Val Pro Leu Thr
1 5

<210> 66
<211> 9
<212> PRT
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<220>
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<400> 66

His Glu Ser Thr Leu Val Pro Leu Thr
1 5

<210> 67
<211> 9
<212> PRT
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<220>
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<400> 67

His Gln Ser Ser Lys Val Pro Leu Thr
1 5

<210> 68
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 68

Ile Gln Thr Ser Leu Val Pro Leu Thr
1 5

<210> 69
<211> 9
<212> PRT
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<220>
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<400> 69

Ile Gln Ala Ala Leu Val Pro Leu Thr
1 5

<210> 70
<211> 9
<212> PRT
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<220>
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<400> 70

Leu Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 71
<211> 9
<212> PRT
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<220>
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<400> 71

Leu Glu Thr Ser Arg Val Pro Leu Thr
1 5

<210> 72
<211> 9
<212> PR1
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<220>
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<400> 72

Leu Ala Ser Ser His Val Pro Leu Thr
1 5

<210> 73
<211> 9
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<220>
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<400> 73

Leu Asn Ser Thr Thr Val Pro Leu Thr
1 5

<210> 74
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 74

Leu Gln Ser Lys Ser Val Pro Leu Thr
1 5

<210> 75
<211> 9
<212> PRT
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<220>
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<400> 75

Leu Gln Ser Val Arg Val Pro Leu Thr
1 5

<210> 76
<211> 9
<212> PRT
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<220>
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<400> 76

Leu Gln Ser Ser Leu Val Pro Leu Thr
1 5

<210> 77
<211> 9
<212> PRT
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<220>
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<400> 77

Leu Gln Thr Gly Arg Val Pro Leu Thr
1 5

<210> 78
<211> 9
<212> PRT
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<220>
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<400> 78

Leu Gln Thr Ser Phe Val Pro Leu Thr
1 5

<210> 79
<211> 9
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<220>
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<400> 79

Leu Gln Thr Ser Asn Val Pro Leu Thr
1 5

<210> 80
<211> 9

<212> PRT
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<220>
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<400> 80

Leu Gln Thr Thr Arg Val Pro Leu Thr
1 5

<210> 81
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 81

Leu Ser Ser Thr Phe Val Pro Leu Thr
1 5

<210> 82
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 82

Leu Ser Ser Thr His Val Pro Leu Thr
1 5

<210> 83
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 83

Leu Thr Ser Ser Ala Val Pro Leu Thr
1 5

<210> 84
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 84

Leu Val Ser Ser Leu Val Pro Leu Thr
1 5

<210> 85
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 85

Met Glu Thr Ala Asn Val Pro Leu Thr
1 5

<210> 86
<211> 9
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<213> Artificial Sequence

<220>
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<400> 86

Met Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 87
<211> 9
<212> PRT
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<220>
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<400> 87

Met Gln Ser Ser Leu Val Pro Leu Thr
1 5

<210> 88
<211> 9
<212> PRT
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<220>
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<400> 88

Met Gln Thr Ser Lys Val Pro Leu Thr
1 5

<210> 89
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 89

Ser Gln Ala Arg Met Val Pro Leu Thr
1 5

<210> 90
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 90

Ser Gln Ala Ser Arg Val Pro Leu Thr
1 5

<210> 91
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 91

Thr Gln Ser Thr Gln Val Pro Leu Thr
1 5

<210> 92
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 92

Val Cys Ala Thr Phe Val Pro Leu Thr

1 5

<210> 93
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 93

Val Gln Ser Ser Ala Val Pro Leu Thr
1 5

<210> 94
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 94

Val Gln Thr Ser Leu Val Pro Leu Thr
1 5

<210> 95
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 95

Val Gln Thr Ser Val Val Pro Leu Thr
1 5

<210> 96
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 96

Val Gln Thr Thr Ala Val Pro Leu Thr
1 5

<210> 97
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 97

Leu Gln Thr Ala Arg Val Pro Leu Thr
1 5

<210> 98
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 98

Phe Gln Gly Ser Leu Leu Pro Leu Ser
1 5

<210> 99
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 99

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 100
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (1)..(5)
<223> Xaa denotes any amino acid residue

<400> 100

Xaa Xaa Xaa Xaa Xaa Arg Ser Ile Asp Ala Met Asp Tyr

1 5 10

<210> 101
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (9)..(13)
<223> Xaa denotes any amino acid residue

<400> 101

Arg Gln Leu Gly Leu Arg Ser Ile Xaa Xaa Xaa Xaa Xaa
1 5 10

<210> 102
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (5)..(9)
<223> Xaa denotes any amino acid residue

<400> 102

Arg Gln Leu Gly Xaa Xaa Xaa Xaa Xaa Ala Met Asp Tyr
1 5 10

<210> 103
<211> 459
<212> DNA
<213> Mus musculus

<400> 103
tgggcagact taccattctc attcctgctg ctgattgtcc ctgcatatgt cttgtcccaa 60

gttactctaa aagagtctgg ccctgggata ttgaagccct cacagaccct cagtctgact 120

tgttctctct ctgggttttc actgagcact tctggtatgg gtgtaggctg gtttcgtcag 180

ccttcaggga agggtctgga gtggctggca cacatttggt gggatgatga taagtcctat 240

aatccatccc tgaagagccg gctcacaatc tccaagtata cctctagaaa ccaggttttc 300

ctcacgatca ccagtgtgga cactgcagat actgccactt actattgtgc tcgaagacaa 360

ctcggactaa gatcaattga tgctatggac tactggggtc aaggaacctc agtcaccgtc 420

tcctcagcca aaacgacacc cccatctgtc tatccactg 459

<210> 104
<211> 435
<212> DNA
<213> Mus musculus

<400> 104
agattgcctg ttaggctgtt ggtgctgatg ttctggattc ctgcttccac cagtgatgtt 60

ttgatgaccc aaactcctct ctccctgcct gtcagtcttg gagatcaagc ctccatctct 120

tgcagatcta gtcagagcat tctacatagt aatggaaaca cctatttaga gtggtacctg 180

cagaaaccag gccagtctcc aaagctcctg atctacaaag tttccaaccg attttctggg 240

gtcccagaca ggttcagtgg cagtggatca gggacagatt tcacactcaa gatcagcaga 300

gtggaggctg aggatctggg agtttattac tgttttcaag gttcacttgt tccgctcacg 360

ttcggtgctg ggaccaagct ggagctgaaa cgggctgatg ctgcaccaac tgtatccatc 420

ttcccaccat ccagt 435

<210> 105
<211> 123
<212> PRT
<213> Mus musculus

<400> 105

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Tyr Thr Ser Arg Asn Gln Val
65 70 75 80

Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 106
<211> 120
<212> PRT
<213> Homo sapiens

<400> 106

Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Arg Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Arg Ile Asp Trp Asp Asp Asp Lys Phe Tyr Ser Thr Ser
50 55 60

Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ile Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 107
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic antibody

<400> 107

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Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
            20                  25                  30

Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120
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<210> 108
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic antibody

<400> 108

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Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Leu Leu Lys Pro Thr Gln
1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
            20                  25                  30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
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65 70 75 80

Val Leu Thr Ile Thr Asn Val Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Arg Gln Leu Gly Thr Arg Gly Thr Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 109
<211> 112
<212> PRT
<213> Mus musculus

<400> 109

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser Leu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 110
<211> 112
<212> PRT
<213> Homo sapiens

<400> 110

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Ser Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Ile His Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 111
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic antibody

<400> 111

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Leu Gln Thr
85 90 95

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 112
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic antibody

<400> 112

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Leu Gln Thr
85 90 95

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 113
<211> 369
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<400> 113
caggtgaccc tgaaggagtc tggccctgcc ctggtgaagc ccacccagac cctgaccctg 60

acctgcacct tctctggctt cagcctgagc acctctggca tgggcgtggg ctggatccgg 120

cagccccctg gcaaggccct ggagtggctg gcccacatct ggtgggacga cgacaagtcc 180

tacaacccca gcctgaagag ccggctgacc atcagcaagg acaccagcaa gaaccaggtg 240

gtgctgacca tgaccaacat ggaccctgtg gacacagcca cctactactg tgcccggcgg 300

cagctgggcc tgcggagcat tgatgccatg gactactggg gccagggcac cacagtgaca 360

gtgtccagc 369

<210> 114
<211> 369
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<400> 114
caggtgaccc tgaaggagtc tggccctgcc ctggtgaagc ccacccagac cctgaccctg 60

acctgcaccc tgtctggctt cagcctgagc acctctggca tgggcgtggg ctggatccgg 120

cagccccctg gcaaggccct ggagtggctg gcccacatct ggtgggacga cgacaagtcc 180

tacaacccca gcctgaagag ccggctgacc atcagcaagg acaccagcaa gaaccaggtg 240

gtgctgacca tgaccaacat ggaccctgtg gacacagcca cctactactg tgcccggcgg 300

cagctgggcc tgcggagcat tgatgccatg gactactggg gccagggcac cacagtgaca 360

gtgtccagc 369

<210> 115
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<400> 115
gatgtggtga tgacccagag ccccctgtcc ctgcctgtga cccctggcga gcctgccagc 60

atctcctgcc ggagctccca gagcatcctg cactccaatg gcaacaccta cctggagtgg 120

tacctgcaga agcctggcca gagcccccag ctgctgatct acaaggtgtc caaccggttc 180

tccggcgtgc ctgaccggtt cagcggctcc ggcagcggca cagacttcac cctgaagatc 240

agccgggtgg aggctgagga tgtgggcgtc tactactgct tccagggcag cctggtgccc 300

ctgacctttg gccagggcac caagctggag atcaag 336

<210> 116
<211> 482
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<400> 116

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
1 5 10 15

Thr Val Ala Gln Ala Ala Leu Glu Gln Val Thr Leu Lys Glu Ser Gly
20 25 30

Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe
35 40 45

Ser Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly Trp Ile Arg
50 55 60

Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala His Ile Trp Trp Asp
65 70 75 80

Asp Asp Lys Ser Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser
85 90 95

Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp
100 105 110

Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Arg Gln Leu Gly Leu
115 120 125

Arg Ser Ile Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr
130 135 140

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
145 150 155 160

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
165 170 175

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
180 185 190

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
195 200 205

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
210 215 220

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Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
225                 230                 235                 240

Val Asp Lys Arg Val Glu Pro Lys Ser Cys Thr Ser Gly His His His
                245                 250                 255

His His His Gly Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly
            260                 265                 270

Gly Pro Phe Val Cys Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln
        275                 280                 285

Pro Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
    290                 295                 300

Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser
305                 310                 315                 320

Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr
                325                 330                 335

Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp
            340                 345                 350

Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala
        355                 360                 365

Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly
    370                 375                 380

Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser
385                 390                 395                 400

Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn
                405                 410                 415

Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro
            420                 425                 430

Tyr Val Phe Gly Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp
        435                 440                 445

Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala
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450 455 460

Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys
465 470 475 480

Glu Ser

<210> 117
<211> 481
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<400> 117

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
1 5 10 15

Thr Val Ala Gln Ala Ala Leu Glu Gln Val Thr Leu Lys Glu Ser Gly
20 25 30

Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Leu
35 40 45

Ser Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly Trp Ile Arg
50 55 60

Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala His Ile Trp Trp Asp
65 70 75 80

Asp Asp Lys Ser Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser
85 90 95

Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp
100 105 110

Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Arg Gln Leu Gly Leu
115 120 125

Arg Ser Ile Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr
130 135 140

Val Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
145 150 155 160

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Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
                165                 170                 175

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
            180                 185                 190

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
        195                 200                 205

Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr
    210                 215                 220

Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val
225                 230                 235                 240

Asp Lys Arg Val Glu Pro Lys Ser Cys Thr Ser Gly His His His His
                245                 250                 255

His His Gly Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly Gly
            260                 265                 270

Pro Phe Val Cys Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro
        275                 280                 285

Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
    290                 295                 300

Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu
305                 310                 315                 320

Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu
                325                 330                 335

Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu
            340                 345                 350

Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr
        355                 360                 365

Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu
    370                 375                 380

Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln
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385 390 395 400

Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn Phe
405 410 415

Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Tyr
420 425 430

Val Phe Gly Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp Lys
435 440 445

Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala Thr
450 455 460

Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys Glu
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<222> (81)..(82)
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ccatggcmnn mnnmnnmnnm nngcccagct g 91

<210> 134
<211> 330
<212> PRT
<213> Homo sapiens

<400> 134

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255
```

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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
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<210> 135
<211> 326
<212> PRT
<213> Homo sapiens

<400> 135

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Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125
```

```
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
            180                 185                 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320

Ser Leu Ser Pro Gly Lys
                325
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<210> 136
<211> 327
<212> PRT
<213> Homo sapiens

<400> 136

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                  10                  15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240
```

```
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245                 250                 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320

Leu Ser Leu Ser Leu Gly Lys
                325
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<210> 137
<211> 326
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<400> 137

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95
```

```
Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
           100                 105                 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
           180                 185                 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
           260                 265                 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320

Ser Leu Ser Pro Gly Lys
                325
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<210> 138
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic polypeptide

<400> 138

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Leu Leu Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Thr Ile Thr Asn Val Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Arg Gln Leu Gly Thr Arg Gly Thr Asp Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
165 170 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190

```
Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val
        195                 200                 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys
    210                 215                 220

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp
            260                 265                 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
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Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
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<210> 139
<211> 1407
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

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tgcaccctgt ctggcttcag cctgagcacc tctggcatgg gcgtgggctg gttccggcag 180

ccccctggca agggcctgga gtggctggcc cacatctggt gggacgacga caagtcctac 240

aaccccagcc tgaagagccg gctgaccatc agcaaggaca ccagcaagaa ccaggtggtg 300

ctgaccatca ccaacgtgga ccctgtggac acagccacct actactgtgc ccggcggcag 360

ctgggcacta gggggacgga tgccatggac tactggggcc agggcaccac agtgacagtg 420

tccagcgcat ccaccaaggg cccatccgtc ttccccctgg cgccctgctc caggagcacc 480

tccgagagca cagccgccct gggctgcctg gtcaaggact acttccccga accggtgacg 540

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gtcttcctgt tccccccaaa acccaaggac actctcatga tctcccggac ccctgaggtc 840

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gatggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagtt caacagcacg 960

ttccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaacgg caaggagtac 1020

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aagaaccagg tcagcctgac ctgcctggtc aaaggcttct accccagcga catcgccgtg 1200

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc catgctggac 1260

tccgacggct ccttcttcct ctacagcaag ctaaccgtgg acaagagcag gtggcagcag 1320

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<213> Artificial Sequence

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<400> 140

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Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Leu Gln Thr
85 90 95

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 141
<211> 720
<212> DNA
<213> Artificial Sequence

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<223> Synthetic oligonucleotide

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atctcctgcc ggagctccca gagcatcctg cactccaatg gcaacaccta cctggagtgg 180

tacctgcaga agcctggcca gagcccccag ctgctgatct acaaggtgtc caaccggttc 240

tccggcgtgc ctgaccggtt cagcggctcc ggcagcggca cagacttcac cctgaagatc 300

agccgggtgg aggctgagga tgtgggcgtc tactactgcc ttcagactac tcgtgtgccc 360

ctgacctttg gccagggcac caagctggag atcaagcgta cggtggctgc accatctgtc 420

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg 480

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tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc 600

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa 660

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag 720

<210> 142
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 142

Arg Gln Leu Gly Lys Leu Ala Leu Asp Ala Met Asp Tyr
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<210> 143
<211> 13
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide

<400> 143

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 144
<211> 13
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide

<400> 144

Arg Gln Leu Gly Gln Arg Gln Thr Asp Ala Met Asp Tyr
1 5 10

<210> 145
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 145

Arg Ala Ile Gln Pro Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 146
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 146

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala His Thr Arg
1 5 10

<210> 147
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 147

Arg Gln Leu Gly Gln Pro Ser Val Asp Ala Met Asp Tyr
1 5 10

<210> 148
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 148

Arg Gln Leu Gly Phe Gln Ser Thr Asp Ala Met Asp Tyr
1 5 10

<210> 149
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 149

Arg Gln Leu Gly Gln Ala Gly His Asp Ala Met Asp Tyr
1 5 10

<210> 150
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 150

Arg Gln Leu Gly Asp Asn Val Ala Asp Ala Met Asp Tyr
1 5 10

<210> 151

<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 151

Arg Gln Leu Gly Met Ala Thr Pro Asp Ala Met Asp Tyr
1 5 10

<210> 152
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 152

Arg Gln Leu Gly Ala His Trp Leu Asp Ala Met Asp Tyr
1 5 10

<210> 153
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 153

Arg Gln Leu Gly Pro Glu Pro Gln Asp Ala Met Asp Tyr
1 5 10

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 June 2007 (07.06.2007)

PCT

(10) International Publication Number
WO 2007/064972 A2

(51) International Patent Classification:
C07K 16/00 (2006.01)

(21) International Application Number:
PCT/US2006/046148

(22) International Filing Date:
30 November 2006 (30.11.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/740,866 30 November 2005 (30.11.2005) US
60/778,950 3 March 2006 (03.03.2006) US

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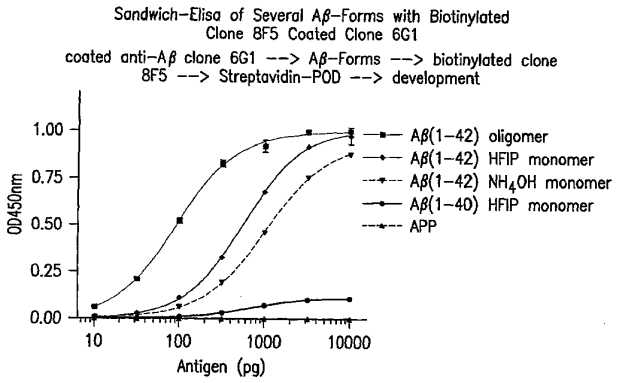
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: MONOCLONAL ANTIBODIES AND USES THEREOF



	Aβ(1-42) oligomer	Aβ(1-42) HFIP monomer	Aβ(1-42) NH_4OH monomer	Aβ(1-40) HFIP monomer
BOTTOM	−0.001218	0.004304	0.006727	0.004288
TOP	0.9926	1.002	0.9292	0.1116
LOGEC50	1.958	2.745	3.003	2.825
HILLSLOPE	1.215	1.270	1.204	1.375
EC50	90.74	555.8	1007	667.8

(57) Abstract: The subject invention relates to monoclonal antibodies (e.g., 8F5 and 8C5) that may be used, for example, in the prevention, treatment and diagnosis of Alzheimer's Disease or other neurodegenerative disorders.

WO 2007/064972 A2

Published:
— *without international search report and to be republished upon receipt of that report*

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MONOCLONAL ANTIBODIES AND USES THEREOF

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to monoclonal antibodies (e.g., 8F5 and 8C5) that may be used, for example, in the prevention, treatment and diagnosis of Alzheimer's Disease or other neurodegenerative disorders.

Background Information

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by a progressive loss of cognitive abilities and by characteristic neuropathological features comprising amyloid deposits, neurofibrillary tangles and neuronal loss in several regions of the brain see Hardy and Selkoe (Science 297, 353 (2002); Mattson (Nature 431, 7004 (2004). The principal constituents of amyloid deposits are amyloid beta-peptides (Aβ), with the 42 amino acid-long type (Aβ1-42) being the most prominent.

In particular, amyloid β(1-42) protein is a polypeptide having 42 amino acids which is derived from the amyloid precursor protein (APP) by proteolytic processing. This also includes, in addition to human variants, isoforms of the amyloid β(1-42) protein present in organisms other than humans, in particular, other mammals, especially rats. This protein, which tends to polymerize in an aqueous environment, may be present in very different molecular forms.

A simple correlation of the deposition of insoluble protein with the occurrence or progression of dementia disorders such as, for example, Alzheimer's disease, has proved to be unconvincing (Terry et al., Ann. Neurol. 30. 572-580 (1991); Dickson et al., Neurobiol. Aging 16, 285-298 (1995)). In contrast, the loss of synapses and cognitive

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5412

perception seems to correlate better with soluble forms of Aβ(1-42)(Lue et al., Am. J. Pathol. 155, 853-862 (1999); McLean et al., Ann. Neurol. 46, 860-866 (1999)).

Although polyclonal and monoclonal antibodies have been raised in the past against Aβ(1-42), none have proven to produce the desired therapeutic effect without also causing serious side effects in animals and/or humans. For example, passive immunization results from preclinical studies in very old APP23 mice which received a N-terminal directed anti-Aβ(1-42) antibody once weekly for 5 months indicate therapeutically relevant side effect. In particular, these mice showed an increase in number and severity of microhemorrhages compared to saline-treated mice (Pfeifer et al., Science 2002 298:1379). A similar increase in hemorrhage was recently also described for very old (>24 months) Tg2576 and PDAPP mice (Wilcock et al., J Neuroscience 2003, 23: 3745-51; Racke et al., J Neuroscience 2005, 25:629-636). In both strains, injection of anti-Aβ(1-42) resulted in a significant increase of microhemorrhages. Thus, a tremendous therapeutic need exists for the development of biologics that prevent or slow down the progression of the disease without inducing negative and potentially lethal effects on the human body. Such need is particularly evident in view of the increasing longevity of the general population and, with this increase, an associated rise in the number of patents annually diagnosed with Alzheimer's Disease. Further, such antibodies will allow for proper diagnosis of Alzheimer's Disease in a patient experiencing symptoms thereof, a diagnosis which can only be confirmed upon autopsy at the present time. Additionally, the antibodies will allow for the elucidation of the biological properties of the proteins and other biological factors responsible for this debilitating disease.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5413

All patents and publications referred to herein are hereby incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention includes an isolated antibody that binds with greater specificity to an amyloid beta (Aβ) protein globulomer than to an amyloid beta protein monomer. Thus, preferential binding is observed. The antibody may be, for example, a monoclonal antibody such as 8F5 or 8C5. The ratio of binding specificity to the globulomer versus the monomer is at least 1.4. In particular, the ratio is preferably at least about 1.4 to at least about 16.9. (A ratio of 1.0-17.5 including the endpoints) is also considered to fall within the scope of the present invention as well as decimal percentages thereof. For example, 1.1, 1.2, 1.3, …, 2.0, 2.1, 2.2…,17.1, 17.2, 17.3, 17.4, 17.5 as well as all full integers in between and percentages thereof are considered to fall within the scope of the present invention.) The amyloid beta protein monomer may be, for example, Aβ(1-42) monomer or Aβ(1-40) monomer.

Further, the present invention also encompasses a monoclonal antibody (referred to herein as "8F5") produced by a hybridoma having American Type Culture Collection designation number PTA-7238 as well as the hybridoma that produces this monoclonal antibody (i.e., 8F5). Also, the present invention includes a monoclonal antibody (referred to herein as "8C5") produced by a hybridoma having American Type Culture Collection designation number PTA-7407 as well as the hybridoma that produces this monoclonal antibody (i.e., 8C5).

Additionally, the present invention includes a monoclonal antibody comprising a variable heavy chain encoded by SEQ ID NO:1. This antibody may be murine, human or humanized.

Further, the present invention includes a monoclonal antibody comprising a variable light chain encoded by SEQ ID NO:2. This antibody may also be murine, human or humanized. The antibody may further comprise a variable light heavy chain encoded by SEQ ID NO:1 and may be human or humanized.

Moreover, the present invention includes a monoclonal antibody comprising SEQ ID NO:3. The antibody may be murine, human or humanized.

Further, the present invention encompasses a monoclonal antibody comprising SEQ ID NO:4. This antibody may be murine, human or humanized. This antibody may further comprise SEQ ID NO:3 and may be murine, human or humanized.

Additionally, the present invention includes a monoclonal antibody comprising a variable heavy chain encoded by SEQ ID NO:11. This antibody may be murine, human or humanized.

Further, the present invention includes a monoclonal antibody comprising a variable light chain encoded by SEQ ID NO:12. This antibody may also be murine, human or humanized. The antibody may further comprise a variable heavy chain encoded by SEQ ID NO:11 and may be human or humanized.

Moreover, the present invention includes a monoclonal antibody comprising SEQ ID NO:19. The antibody may be murine, human or humanized.

Further, the present invention encompasses a monoclonal antibody comprising SEQ ID NO:20. This antibody may be murine, human or humanized. This antibody may further comprise SEQ ID NO:19 and may be murine, human or humanized.

The present invention also includes an isolated antibody which binds with greater specificity to an amyloid beta protein globulomer than to an amyloid beta protein fibril. This antibody may be, for example, monoclonal and may be the monoclonal antibody produced by the hybridoma having American Type Culture Collection designation number PTA-7243 or the

hybridoma having American Type Culture Collection PTA-7407. The hybridomas producing these monoclonal antibodies also fall within the scope of the present invention.

Further, the present invention includes an antibody in which at least one of the complementarity determining regions (CDRs) of the variable heavy chain is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

Moreover, the present invention also includes an antibody in which at least one of the CDRs of the variable light chain is selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10. This antibody may further comprise at least one CDR of the variable heavy chain selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

The present invention also includes an antibody in which at least one of the CDRs of the variable heavy chain is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

Further, the present invention also encompasses an antibody in which at least one of the CDRs of the variable light chain is selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18. This antibody may further comprises at least one CDR of the variable heavy chain selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

Additionally, the present invention encompasses a method of treating or preventing Alzheimer's Disease in a patient in need of the treatment or prevention. This method comprises administering any one or more of the isolated antibodies described above to the patient in an amount sufficient to effect the treatment or prevention.

The isolated antibody may be administered, for example, *via* a route selected from the group consisting of intramuscular

administration, intravenous administration and subcutaneous administration.

The present invention also includes a method of diagnosing Alzheimer's Disease in a patient suspected of having this disease. This method comprises the steps of: 1) isolating a biological sample from the patient; 2) contacting the biological sample with at least one of the antibodies described above for a time and under conditions sufficient for formation of antigen/antibody complexes; and 3) detecting presence of the antigen/antibody complexes in said sample, presence of the complexes indicating a diagnosis of Alzheimer's Disease in the patient. The antigen may be, for example, a globulomer or a portion or fragment thereof which has the same functional properties as the full globulomer (e.g., binding activity).

Further, the present invention includes another method of diagnosing Alzheimer's Disease in a patient suspected of having this disease. This method comprises the steps of: 1) isolating a biological sample from the patient; 2) contacting the biological sample with an antigen for a time and under conditions sufficient for the formation of antibody/antigen complexes; 3) adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein the conjugate comprises one of the antibodies described above, attached to a signal generating compound capable of generating a detectable signal; and 4) detecting the presence of an antibody which may be present in the biological sample, by detecting a signal generated by the signal generating compound, the signal indicating a diagnosis of Alzheimer's Disease in the patient. The antigen may be a globulomer or a portion or fragment thereof having the same

functional properties as the full globulomer (e.g., binding activity).

The present invention includes an additional method of diagnosing Alzheimer's Disease in a patient suspected of having Alzheimer's Disease. This method comprises the steps of: 1) isolating a biological sample from the patient; 2) contacting the biological sample with anti-antibody, wherein the anti-antibody is specific for one of the antibodies described above, for a time and under conditions sufficient to allow for formation of anti-antibody/antibody complexes, the complexes containing antibody present in the biological sample; 2) adding a conjugate to the resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to bound antibody, wherein the conjugate comprises an antigen, which binds to a signal generating compound capable of generating a detectable signal; and 3) detecting a signal generated by the signal generating compound, the signal indicating a diagnosis of Alzheimer's Disease in the patient.

Further, the present invention includes a composition comprising any one or more of the antibodies described above (e.g., 8F5 and 8C5).

The present invention includes another method of preventing or treating Alzheimer's Disease in a patient in need of such prevention or treatment. This method comprises the step of administering the composition described directly above to the patient in an amount sufficient to effect the prevention or treatment.

Additionally, the present invention encompasses a vaccine comprising at least one of the antibodies described above and a pharmaceutically acceptable adjuvant.

Moreover, the present invention includes a further method of preventing or treating Alzheimer's Disease in a patient in

need of such prevention or treatment. This method comprises the step of administering the vaccine noted above to the patient in an amount sufficient to effect the prevention or treatment.

Further, the present invention encompasses a method of identifying compounds suitable for active immunization of a patient predicted to develop Alzheimer's Disease. This method comprises: 1) exposing one or more compounds of interest to one or more of the antibodies described above for a time and under conditions sufficient for the one or more compounds to bind to the antibody or antibodies; 2) identifying those compounds which bind to the antibody or antibodies, the identified compounds to be used in active immunization in a patient predicted to develop Alzheimer's Disease.

Also, the present invention includes a kit comprising: a) at least one of the isolated antibodies described above and b) a conjugate comprising an antibody attached to a signal-generating compound, wherein the antibody of the conjugate is different from the isolated antibody. The kit may also include a package insert with instructions as to how the components of the kit are to be utilized.

The present invention also encompasses a kit comprising: a) an anti-antibody to one of the antibodies described above and b) a conjugate comprising an antigen attached to a signal-generating compound. The antigen may be a globulomer or a fragment or portion thereof having the same functional characteristics as the globulomer (e.g., binding activity). Again, the kit may also include a package insert with instructions as to how the components of the kit are to be utilized.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the selectivity of 8F5 for globulomers versus Aß(1-42) monomers, Aß(1-40) and sAPP. Selectivity factors for 8F5 can be calculated as ratios between EC50 values (versus Aß(1-42) monomer in HFIP: 555.8/90.74 = 6.1; versus Aß(1-42) monomer in NH_4OH: 1007/90.74 = 11.1; versus Aß(1-40) monomer: 667.8/90.74 = 7.4 versus sAPP: >100)

Figure 2 illustrates SDS-PAGE analysis of fibril bound heavy and light chain antibodies (lanes 4, 6, 8) and corresponding non-bound free fractions (lanes 3, 5, 7) in the supernatants.

Figure 3 illustrates Aß42 and Aß40 content in CSF samples from patients with Mild Cognitive Impairment (MCI, left) or Alzheimer's disease (AD, right). In both groups, it can be observed that 8F5 picks up a higher proportion of Aß(1-42) and less or an equal amount of Aß(1-40) if compared to a standard antibody 6E10 or compared to direct sample analysis with the same ELISAs.

Figure 4 illustrates novel object recognition index as time spent with unknown versus familiar object in three groups of APP transgenic mice (i.e., 6G1, 8F5, PBS) and one group of non-transgenic litter mates (wild type). The animals (number given below columns) were immunized with monoclonal antibodies 6G1 or 8F5 or treated with vehicle (i.e., phosphate-buffered saline; PBS, and wild type) by once weekly intraperitoneal injection for three weeks. On the day of the last injection, a novel object recognition task was performed. The difference between PBS and wild type groups indicated a cognitive deficit of APP transgenic mice in this paradigm. PBS-injected mice performed at chance level (i.e., not significantly different from 50) while all other mice showed object recognition (t-test; stars). When the performance of antibody-treated APP

transgenic mice was compared with control groups, a significant difference was found versus PBS-treated but not versus wild-type mice (ANOVA with post-hoc t-test; circles) indicating that antibody treatment reversed the cognitive deficit in these APP transgenic mice.

Figure 5(A) illustrates the DNA sequence (SEQ ID NO:1) of the variable heavy chain encoding the monoclonal antibody referred to herein as "8F5", and Figure 5(B) illustrates the DNA sequence (SEQ ID NO:2) of the variable light chain encoding the monoclonal antibody 8F5. (Complementarity determining regions (CDRs) are underlined in each sequence; see also Figure 6.)

Figure 6(A) illustrates the amino acid sequence (SEQ ID NO:3) of the variable heavy chain of monoclonal antibody 8F5, and Figure 6(B) illustrates the amino acid sequence (SEQ ID NO:4) of the variable light chain of monoclonal antibody 8F5. One CDR of the variable heavy chain is represented by the amino acid sequence SYGMS (SEQ ID NO:5). Another CDR of the variable heavy chain is represented by the amino acid sequence ASINSNGGSTYYPDSVKG (SEQ ID NO:6), and another CDR of the variable heavy chain is represented by the amino acid sequence SGDY (SEQ ID NO:7). One CDR of the variable light chain is represented by the amino acid sequence RSSQSLVYSNGDTYLH (SEQ ID NO:8). Another CDR of the variable light chain is represented by the amino acid sequence KVSNRFS (SEQ ID NO:9), and another CDR of the variable light chain is represented by the amino acid sequence SQSTHVPWT (SEQ ID NO:10). All of the above-described CDRs are underlined in Figure 6(A) and 6(B).

Figure 7 shows the binding of antibodies, at different concentrations, to transversal sections of the neocortices of Alzheimer's disease (AD) patients or old APP transgenic mice. In particular, Figure 7(A) illustrates verification of amyloid deposits by Congo Red staining as plaques in brain tissue and

as cerebral amyloid angiopathy (CAA) in brain vessels in the APP transgenic mouse line Tg2576 and in an AD patient (RZ55). Figure 7(B) illustrates that the staining of parenchymal deposits of Aβ (amyloid plaques) in an AD patient (RZ16) occurs only with 6G1 and the commercially available antibody 6E10 while 8F5 and 8C5 show considerably weaker staining. Figure 7(C) illustrates that the strong staining of parenchymal deposits of Aβ (amyloid plaques) in TG2576 mice occurs only with 6G1 and the commercially available antibody 6E10 while 8F5 and 8C5 show considerably weaker staining. Figures 7(D)-(G) illustrate the quantification of the analysis of Aβ plaque staining in the histological images using image analysis. Optical density values (0% = no staining) were calculated from the greyscale values of plaques subtracted by greyscale values of background tissue. (Fig. (D)= binding of 0.7 μg/ml antibody in Tg2576 mice; Fig. (E)= binding of 0.07-0.7 μg/ml antibody in APP/L mice; Fig. (F)= binding of 0.7 μg/ml antibody in an AD patient (RZ55); and Fig. (G)= binding of 0.07-0.7 μg/ml antibody in an AD patient (RZ16)) The differences between staining of the commercially available antibodies 6E10 (starts) and 4G8 (circles) and antibodies 6G1, 8C5 and 8F5 (one asterisk/circle: $p < 0.05$, two asterisks/circles: $p < 0.01$, and three asterisks/circles: $p<0.001$ versus control; post-hoc Bonferroni's t-test after ANOVA with $p<0.001$) were statistically evaluated (Fig. (D) and Fig. (E)). In Figs. (E) and (G), the antibodies 8C5 and 8F5 always showed significantly less staining than the commercially available antibodies 6E10 and 4G8 ($p<0.05$ in post-hoc t-test after $p<0.001$ in ANOVA). Figure (H) illustrates that the strong staining of vascular deposits of Aβ (arrows) occurs only with

6G1 and the commercially available antibody 6E10 while staining with 8F5 or 8C5 was much weaker. A qualitatively similar situation was found in Tg2576 mice (not shown here).

Figure 8 illustrates the selectivity of 8C5 for globulomers versus Aß(1-42) monomers, Aß(1-40) and sAPP. Selectivity factors for 8C5 can be calculated as ratios between EC50 values (versus Aß(1-42) monomer in HFIP: 2346/568.2 = 4.1; versus Aß(1-42) monomer in NH_4OH: >100; versus Aß(1-40) monomer: >100; versus sAPP: >100)

Figure 9(A) illustrates the nucleotide sequence (SEQ ID NO:11) encoding the heavy chain of 8C5 and Figure 9(B) illustrates the nucleotide sequence (SEQ ID NO:12) encoding the light chain of 8C5. The nucleotide sequences encoding the corresponding CDRs, noted in Figure 10(A) and 10(B), are underlined.

Figure 10(B) illustrates the amino acid sequence (SEQ ID NO:19) of the variable heavy chain of monoclonal antibody 8C5, and Figure 10(B) illustrates the amino acid sequence (SEQ ID NO:20) of the variable light chain of monoclonal antibody 8F5. One CDR of the variable heavy chain is represented by the amino acid sequence SYGMS (SEQ ID NO:13). Another CDR of the variable heavy chain is represented by the amino acid sequence SIKNNGGSTYYPDSLKG (SEQ ID NO:14), and another CDR of the variable heavy chain is represented by the amino acid sequence SGDY (SEQ ID NO:15). One CDR of the variable light chain is represented by the amino acid sequence RSSQSLVHSNGDTFLH (SEQ ID NO:16). Another CDR of the variable light chain is represented by the amino acid sequence KVSNRFS (SEQ ID NO:17), and another CDR of the variable light chain is represented by

the amino acid sequence SQSIHVPWT (SEQ ID NO:18). All of the above-described CDRs are underlined in Figure 10(A) and 10(B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a monoclonal antibody, referred to herein as "8F5" as well as other related antibodies (e.g., 8C5). These antibodies may be used, for example, in the diagnosis, prevention and treatment of Alzheimer's Diseases and other neurodegenerative disorders.

Monoclonal antibody 8F5 as well as monoclonal antibody 8C5 have many interesting properties which allow them to be extremely interesting therapeutic candidates as well as extremely useful diagnostic candidates. For example, monoclonal antibodies 8F5 and 8C5 have preferential binding for Aβ(1-42) globulomers as compared with monomers or fibrils.

The term "Aβ(X-Y)" herein refers to the amino acid sequence from amino acid position X to amino acid position Y of the human amyloid β protein including both X and Y and, in particular, refers to the amino acid sequence from amino acid position X to amino acid position Y of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA or any of its naturally occurring variants, in particular, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start position of the Aβ peptide, including both position X and position Y or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation. An "additional" amino acid substitution is defined herein as any deviation from the canonical sequence that is not found in nature.

More specifically, the term "Aβ(1-42)" herein refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the human amyloid β protein including both 1 and 42 and, in particular, refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA (corresponding to amino acid positions 1 to 42) or any of its naturally occurring variants. Such variants may be, for example, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβ peptide, including both 1 and 42 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation. Likewise, the term "Aβ(1-40)" here refers to the amino acid sequence from amino acid position 1 to amino acid position 40 of the human amyloid β protein including both 1 and 40 and refers, in particular, to the amino acid sequence from amino acid position 1 to amino acid position 40 of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV or any of its naturally occurring variants. Such variants include, for example, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), and D23N ("Iowa") wherein the numbers are relative to the start position of the Aβ peptide, including both 1 and 40 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation.

The term "Aβ(X-Y) globulomer" (also known as "Aβ(X-Y) globular oligomer") herein refers to a soluble, globular, non-

covalent association of Aβ (X-Y) peptides, as defined above, possessing homogeneity and distinct physical characteristics. The Aβ(X-Y) globulomers are stable, non-fibrillar, oligomeric assemblies of Aβ (X-Y) peptides which are obtainable by incubation with anionic detergents. In contrast to monomer and fibrils, these globulomers are characterized by defined assembly numbers of subunits (e.g., early assembly forms, n=3-6, oligomers A", and late assembly forms, n=12- 14, " oligomers B", as described in PCT International Application Publication No. WO 04/067561). The globulomers have a 3-dimensional globular type structure ("molten globule", see Barghorn et al., 2005, J Neurochem, 95, 834-847). They may be further characterized by one or more of the following features:

- cleavability of N-terminal amino acids X-23 with promiscuous proteases (such as thermolysin or endoproteinase GluC) yielding truncated forms Aß(X-Y) globulomers;
- non-accessibility of C-terminal amino acids 24-Y with promiscuous proteases and antibodies; and
- truncated forms of these Aß(X-Y) globulomers maintain the 3-dimensional core structure of the globulomers with a better accessibility of the core epitope Aß(20-Y) in its globulomer conformation.

According to the invention and, in particular, for the purpose of assessing the binding affinities of the antibodies of the present invention, the term "Aβ(X-Y) globulomer" herein refers to a product which is obtainable by a process as described in International Application Publication No. WO 04/067561, which is incorporated herein in its entirety by reference. The process comprises unfolding a natural, recombinant or synthetic Aβ (X-Y) peptide or a derivative

thereof; exposing the at least partially unfolded Aβ (X-Y) peptide or derivative thereof to a detergent, reducing the detergent action and continuing incubation.

For the purpose of unfolding the peptide, hydrogen bond-breaking agents such as, for example, hexafluoroisopropanol (HFIP) may be allowed to act on the protein. Times of action of a few minutes, for example about 10 to 60 minutes, are sufficient when the temperature of action is from about 20 to 50°C and, in particular, about 35 to 40°C. Subsequent dissolution of the residue evaporated to dryness, preferably in concentrated form, in suitable organic solvents miscible with aqueous buffers such as, for example, dimethyl sulfoxide (DMSO), results in a suspension of the at least partially unfolded peptide or derivative thereof which can be used subsequently. If required, the stock suspension may be stored at low temperature, for example, at about -20°C for an interim period.

Alternatively, the peptide or the derivative thereof may be taken up in slightly acidic, preferably aqueous, solution, for example, a solution of about 10 mM aqueous HCl. After an incubation time of approximately a few minutes, insoluble components are removed by centrifugation. A few minutes at 10,000 g is expedient. These method steps are preferably carried out at room temperature, i.e., a temperature in the range of from 20 to 30°C. The supernatant obtained after centrifugation contains the Aβ (X-Y) peptide or a derivative thereof and may be stored at low temperature, for example at about -20°C, for an interim period.

The following exposure to a detergent relates to oligomerization of the peptide or the derivative thereof to give the intermediate type of oligomers (in International Application Publication No. WO 04/067561 referred to as

oligomers A). For this purpose, a detergent is allowed to act on the, optionally, at least partially unfolded peptide or derivative thereof until sufficient intermediate oligomer has been produced. Preference is given to using ionic detergents, in particular, anionic detergents.

According to a particular embodiment, a detergent of the formula (I):

R-X,

is used, in which the radical "R" is unbranched or branched alkyl having from 6 to 20 and preferably 10 to 14 carbon atoms or unbranched or branched alkenyl having from 6 to 20 and preferably 10 to 14 carbon atoms, and the radical "X" is an acidic group or salt thereof with X being preferably selected from among $-COO^-M^+$, $-SO_3^-M^+$ and is, most preferably, $-OSO_3^-M^+$, and M^+ is a hydrogen cation or an inorganic or organic cation preferably selected from alkali metal cations, alkaline earth metal cations and ammonium cations. Most advantageous are detergents of the formula (I) in which R is an unbranched alkyl of which alk-1-yl radicals must be mentioned, in particular. Particular preference is given to sodium dodecyl sulfate (SDS). Lauric acid and oleic acid can also be used advantageously. The sodium salt of the detergent lauroylsarcosin (also known as sarkosyl NL-30 or Gardol®) is also particularly advantageous.

The time of detergent action, in particular, depends on whether, and if yes, to what extent the peptide or derivative thereof subjected to oligomerization has unfolded. If, according to the unfolding step, the peptide or derivative thereof has been treated beforehand with a hydrogen bond-breaking agent (i.e., in particular with hexafluoroisopropanol), times of action in the range of a few hours, advantageously from about 1 to 20 and, in particular, from about 2 to 10 hours, are sufficient when the temperature

of action is about 20 to 50°C and, in particular, from about 35 to 40°C. If a less unfolded or an essentially not unfolded peptide or derivative thereof is the starting point, correspondingly longer times of action are expedient. If the peptide or derivative thereof has been pretreated, for example, according to the procedure indicated above as an alternative to the HFIP treatment or said peptide or derivative thereof is directly subjected to oligomerization, times of action in the range from about 5 to 30 hours and, in particular, from about 10 to 20 hours are sufficient when the temperature of action is from about 20 to 50°C and, in particular, from about 35 to 40°C. After incubation, insoluble components are advantageously removed by centrifugation. A few minutes at 10,000 g is expedient.

The detergent concentration to be chosen depends on the detergent used. If SDS is used, a concentration in the range from 0.01 to 1% by weight, preferably, from 0.05 to 0.5% by weight, for example, of about 0.2% by weight, proves expedient. If lauric acid or oleic acid is used, somewhat higher concentrations are expedient, for example, in a range from 0.05 to 2% by weight, preferably, from 0.1 to 0.5% by weight, for example, of about 0.5% by weight.
The detergent action should take place at a salt concentration approximately in the physiological range. Thus, in particular NaCl concentrations in the range from 50 to 500 mM, preferably, from 100 to 200 mM and, more particularly, at about 140 mM are expedient.

The subsequent reduction of the detergent action and continuation of incubation relates to further oligomerization to give the Aβ(X-Y) globulomer of the invention (in International Application Publication No. WO 04/067561 referred to as oligomer B). Since the composition obtained from the preceding step regularly contains detergent and a

salt concentration in the physiological range, it is then expedient to reduce detergent action and, preferably, also salt concentration. This may be carried out by reducing the concentration of detergent and salt, for example, by diluting expediently with water or a buffer of lower salt concentration, for example, Tris-HCl, pH 7.3. Dilution factors in the range from about 2 to 10, advantageously, in the range from about 3 to 8 and, in particular, of about 4, have proved suitable. The reduction in detergent action may also be achieved by adding substances which can neutralize this detergent action. Examples of these include substances capable of complexing the detergents, like substances capable of stabilizing cells in the course of purification and extraction measures, for example, particular EO/PO block copolymers, in particular, the block copolymer under the trade name Pluronic® F 68. Alkoxylated and, in particular, ethoxylated alkyl phenols such as the ethoxylated t-octylphenols of the Triton® X series, in particular, Triton® X100, 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonate (CHAPS®) or alkoxylated and, in particular, ethoxylated sorbitan fatty esters such as those of the Tween® series, in particular, Tween® 20, in concentration ranges around or above the particular critical micelle concentration, may be equally used.

Subsequently, the solution is incubated until sufficient Aβ(X-Y) globulomer has been produced. Times of action in the range of several hours, preferably, in the range from about 10 to 30 hours and, in particular, in the range from about 15 to 25 hours, are sufficient when the temperature of action is about 20 to 50°C and, in particular, about 35 to 40°C. The solution may then be concentrated and possible residues may be removed by centrifugation. Again, a few minutes at 10,000 g proves expedient. The supernatant obtained after

centrifugation contains an Aβ(X-Y) globulomer as described herein.

An Aβ(X-Y) globulomer can be finally recovered, e.g. by ultrafiltration, dialysis, precipitation or centrifugation. It is further preferred if electrophoretic separation of the Aβ(X-Y) globulomers under denaturing conditions, e.g. by SDS-PAGE, produces a double band (e.g., with an apparent molecular weight of 38/48 kDa for Aβ (1-42)) and especially preferred if upon glutardialdehyde treatment of the oligomers, before separation, these two bands are merged into one. It is also preferred if size exclusion chromatography of the globulomers results in a single peak (e.g., corresponding to a molecular weight of approximately 60 kDa for Aβ (1-42)). Starting from Aβ(1-42) peptide, the process is, in particular, suitable for obtaining Aβ(1-42) globulomers

Preferably, the globulomer shows affinity to neuronal cells and also exhibits neuromodulating effects.

A "neuromodulating effect" is defined as a long-lasting inhibitory effect of a neuron leading to a dysfunction of the neuron with respect to neuronal plasticity.

According to another aspect of the invention, the term "Aβ(X-Y) globulomer" herein refers to a globulomer consisting essentially of Aβ(X-Y) subunits, wherein it is preferred if, on average, at least 11 of 12 subunits are of the Aβ(X-Y) type, more preferred, if less than 10% of the globulomers comprise any non-Aβ(X-Y) peptides and, most preferred, if the content of non-Aβ(X-Y) peptides in the preparation is below the detection threshold. More specifically, the term "Aβ(1-42) globulomer" herein refers to a globulomer comprising Aβ(1-42) units as defined above; the term "Aβ(12-42) globulomer" herein refers to a globulomer comprising Aβ(12-42) units as defined above; and the term "Aβ(20-42) globulomer" herein

refers to a globulomer comprising Aβ(20-42) units as defined above.

The term "cross-linked Aβ(X-Y) globulomer" herein refers to a molecule obtainable from an Aβ(X-Y) globulomer as described above by cross-linking, preferably, chemically cross-linking, more preferably, aldehyde cross-linking and, most preferably, glutardialdehyde cross-linking of the constituent units of the globulomer. In another aspect of the invention, a cross-linked globulomer is essentially a globulomer in which the units are at least partially joined by covalent bonds, rather than being held together by non-covalent interactions only.

The term "Aβ(X-Y) globulomer derivative" herein refers, in particular, to a globulomer that is labelled by being covalently linked to a group that facilitates detection, preferably, a fluorophore, e.g., fluorescein isothiocyanate, phycoerythrin, Aequorea victoria fluorescent protein, Dictyosoma fluorescent protein or any combination or fluorescence-active derivatives thereof; a chromophore; a chemoluminophore, e.g., luciferase, preferably Photinus pyralis luciferase, Vibrio fischeri luciferase, or any combination or chemoluminescence-active derivatives thereof; an enzymatically active group, e.g., peroxidase such as horseradish peroxidase, or an enzymatically active derivative thereof; an electron-dense group, e.g., a heavy metal containing group such as a gold containing group; a hapten, e.g., a phenol derived hapten; a strongly antigenic structure, e.g., peptide sequence predicted to be antigenic such as by the algorithm of Kolaskar and Tongaonkar; an aptamer for another molecule; a chelating group, e.g., hexahistidinyl; a natural or nature-derived protein structure mediating further specific protein-protein interactions, e.g., a member of the fos/jun pair; a magnetic group, e.g., a ferromagnetic group;

or a radioactive group such as a group comprising ^{1}H, ^{14}C, ^{32}P, ^{35}S or ^{125}I or any combination thereof; or to a globulomer flagged by being covalently or by non-covalently linked by high-affinity interaction, preferably, covalently linked to a group that facilitates inactivation, sequestration, degradation and/or precipitation, preferably, flagged with a group that promotes *in vivo* degradation, more preferably, with ubiquitin, where it is particularly preferred if this flagged oligomer is assembled *in vivo*; or to a globulomer modified by any combination of the above. Such labelling and flagging groups and methods for attaching them to proteins are known in the art. Labelling and/or flagging may be performed before, during or after globulomerization. In another aspect of the invention, a globulomer derivative is a molecule obtainable from a globulomer by a labelling and/or flagging reaction. Correspondingly, the term "Aβ(X-Y) monomer derivative" herein refers, in particular, to an Aβ monomer that is labelled or flagged as described for the globulomer.

The term "greater affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer, on the one hand, and antibody-globulomer complex, on the other, is further in favor of the antibody-globulomer complex. Likewise, the term "smaller affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer, on the one hand, and antibody-globulomer complex, on the other, is further in favor of the unbound antibody and unbound globulomer.

The term "Aβ(X-Y) monomer" herein refers to the isolated form of the Aβ(X-Y) peptide, preferably, a form of the Aβ(X-Y) peptide which is not engaged in essentially non-covalent interactions with other Aβ peptides. Practically, the Aβ(X-Y) monomer is usually provided in the form of an aqueous

solution. Preferably, the aqueous monomer solution contains 0.05% to 0.2%, more preferably, about 0.1% NaOH when used, for instance, for determining the binding affinity of the antibody of the present invention. In another preferable situation, the aqueous monomer solution contains 0.05% to 0.2%, more preferably, about 0.1% NaOH. When used, it may be expedient to dilute the solution in an appropriate manner. Further, it is usually expedient to use the solution within 2 hours, in particular, within 1 hour, and, especially, within 30 minutes after its preparation.

The term "fibril" herein refers to a molecular structure that comprises assemblies of non-covalently associated, individual Aβ(X-Y) peptides which show fibrillary structure under the electron microscope, which bind Congo red, exhibit birefringence under polarized light and whose X-ray diffraction pattern is a cross-β structure. The fibril may also be defined as a molecular structure obtainable by a process that comprises the self-induced polymeric aggregation of a suitable Aβ peptide in the absence of detergents, e.g., in 0.1 M HCl, leading to the formation of aggregates of more than 24, preferably, more than 100 units. This process is well known in the art. Expediently, Aβ(X-Y) fibril is used in the form of an aqueous solution. In a particularly preferred embodiment of the invention, the aqueous fibril solution is made by dissolving the Aβ peptide in 0.1% NH_4OH, diluting it 1:4 with 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, followed by readjusting the pH to 7.4, incubating the solution at 37 °C for 20 h, followed by centrifugation at 10000 g for 10 min and resuspension in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4.

The term "Aβ(X-Y) fibril" herein refers to a fibril comprising Aβ(X-Y) subunits where it is preferred if, on average, at least 90% of the subunits are of the Aβ(X-Y) type,

more preferred, if at least 98% of the subunits are of the Aβ(X-Y) type and, most preferred, if the content of non-Aβ(X-Y) peptides is below the detection threshold.

Turning back to 8F5, as evidenced by Figure 1, as well as 8C5 (Figure 8), Aβ(1-42) globulomer-specific antibodies monoclonal antibodies 8F5 and 8C5 recognize predominantly Aβ(1-42) globulomer forms and not standard preparations of Aβ(1-40) or Aβ(1-42) monomers including aggregated Aβ(1-42) in contrast to nonspecific antibodies 6G1 and 6E10. In particular, 8F5 detects Aβ(1-42) globulomers only by native PAGE-western blot and not by SDS-PAGE Western blot analysis indicating binding to a more complex detergent-dissociable intersubunit epitope in the core Aβ(1-42) globulomer structure. An intersubunit epitope is defined as a complex non-linear through space epitope located on at least two subunits. More specifically, dot-blot analysis against various Aβ(1-42) and Aβ(1-40) standard preparations showed significant differences in recognition of Aβ(1-42) globulomer versus non-globulomer Aβ forms (standard Aβ(1-40)/(1-42) monomer preparation, aggregated Aβ(1-42) for specific 8F5 and 8C5 but not for the isoform non-specific antibodies 6G1 and 6E10. The globulomer specificity of 8F5 and 8C5 but not of 6G1 and 6E10, was confirmed by quantifying Aβ(1-42) globulomer, Aβ(1-42) monomer, Aß(1-40) monomer and soluble amyloid precursor protein alpha binding in sandwich ELISAs. Further, since these antibodies access the globulomer after native but not after SDS Western blotting, it is likely that each antibody recognizes a structural non-linear epitope in between subunits in the region of amino acids 20 to 30 of Aβ(1-42). Such specificity for globulomers is important because specifically targeting the globulomer form of Aβ with a globulomer preferential antibody such as, for example, 8F5 or 8C5 will: 1) avoid targeting insoluble amyloid deposits,

binding to which may account for inflammatory side effects observed during immunizations with insoluble Aβ; 2) spare Aβ monomer and APP that are reported to have precognitive physiological functions (Plan et al., J. of Neuroscience 23:5531-5535 (2003); and 3) increase the bioavailability of the antibody, as it would not be shaded or inaccessible through extensive binding to insoluble deposits.

The subject invention also includes isolated nucleotide sequences (or fragments thereof) encoding the variable light and heavy chains of monoclonal antibody 8F5 and 8CD as well as those nucleotide sequences (or fragments thereof) having sequences comprising, corresponding to, identical to, hybridizable to, or complementary to at least about 70% (e.g., 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79%), preferably at least about 80% (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%), and more preferably at least about 90% (e.g, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identity to these encoding nucleotide sequences. (All integers (and portions thereof) between and including 70% and 100% are considered to be within the scope of the present invention with respect to percent identity.) Such sequences may be derived from any source (e.g., either isolated from a natural source, produced via a semi-synthetic route, or synthesized *de novo*). In particular, such sequences may be isolated or derived from sources other than described in the examples (e.g., bacteria, fungus, algae, mouse or human).

In addition to the nucleotide sequences described above, the present invention also includes amino acid sequences of the variable light and heavy chains of monoclonal antibody 8F5 and monoclonal antibody 8C5 (or fragments of these amino acid sequences). Further, the present invention also includes amino acid sequences (or fragments thereof) comprising, corresponding to, identical to, or complementary to at least

about 70%, preferably at least about 80%, and more preferably at least about 90% identity to the amino acid sequences of the proteins of the present invention. (Again, all integers (and portions thereof) between and including 70% and 100% (as recited in connection with the nucleotide sequence identities noted above) are also considered to be within the scope of the present invention with respect to percent identity.)

For purposes of the present invention, a "fragment" of a nucleotide sequence is defined as a contiguous sequence of approximately at least 6, preferably at least about 8, more preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides corresponding to a region of the specified nucleotide sequence.

The term "identity" refers to the relatedness of two sequences on a nucleotide-by-nucleotide basis over a particular comparison window or segment. Thus, identity is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments (or two amino acid sequences). "Percentage of sequence identity" is calculated by comparing two optimally aligned sequences over a particular region, determining the number of positions at which the identical base or amino acid occurs in both sequences in order to yield the number of matched positions, dividing the number of such positions by the total number of positions in the segment being compared and multiplying the result by 100. Optimal alignment of sequences may be conducted by the algorithm of Smith & Waterman, Appl. Math. 2:482 (1981), by the algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the method of Pearson & Lipman, Proc. Natl. Acad. Sci. (USA) 85:2444 (1988) and by computer programs which implement the relevant algorithms (e.g., Clustal Macaw Pileup (http://cmgm.stanford.edu/biochem218/11Multiple.pdf; Higgins

et al., CABIOS. 5L151-153 (1989)), FASTDB (Intelligenetics), BLAST (National Center for Biomedical Information; Altschul et al., Nucleic Acids Research 25:3389-3402 (1997)), PILEUP (Genetics Computer Group, Madison, WI) or GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, Madison, WI). (See U.S. Patent No. 5,912,120.)

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the anti-sense strand of the other DNA segment, under appropriate conditions, to form a double helix. A "complement" is defined as a sequence which pairs to a given sequence based upon the canonic base-pairing rules. For example, a sequence A-G-T in one nucleotide strand is "complementary" to T-C-A in the other strand.

In the double helix, adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of the two DNA segments.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.) The definitions of "complementarity", "identity" and "similarity" are well known to those of ordinary skill in the art.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 amino acids, more preferably at least 8 amino acids, and even more preferably at least 15 amino acids from a polypeptide encoded by the nucleic acid sequence.

Additionally, a nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

The term "hybridization" as used herein is generally used to mean hybridization of nucleic acids at appropriate conditions of stringency as would be readily evident to those skilled in the art depending upon the nature of the probe sequence and target sequences. Conditions of hybridization and washing are well known in the art, and the adjustment of conditions depending upon the desired stringency by varying incubation time, temperature and/or ionic strength of the solution are readily accomplished. See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring harbor Press, Cold Spring harbor, N.Y., 1989, as noted above and incorporated herein by reference. (See also Short Protocols in Molecular Biology, ed. Ausubel et al. and Tijssen, Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993), both incorporated herein by reference.) Specifically, the choice of conditions is

dictated by the length of the sequences being hybridized, in particular, the length of the probe sequence, the relative G-C content of the nucleic acids and the amount of mismatches to be permitted. Low stringency conditions are preferred when partial hybridization between strands that have lesser degrees of complementarity is desired. When perfect or near perfect complementarity is desired, high stringency conditions are preferred. For typical high stringency conditions, the hybridization solution contains 6 X S.S.C., 0.01 M EDTA, 1x Denhardt's solution and 0.5% SDS. Hybridization is carried out at about 68 degrees Celsius for about 3 to 4 hours for fragments of cloned DNA and for about 12 to about 16 hours for total eukaryotic DNA. For moderate stringencies, one may utilize filter pre-hybridizing and hybridizing with a solution of 3 X sodium chloride, sodium citrate (SSC), 50% formamide (0.1 M of this buffer at pH 7.5) and 5 X Denhardt's solution. One may then pre-hybridize at 37 degrees Celsius for 4 hours, followed by hybridization at 37 degrees Celsius with an amount of labeled probe equal to 3,000,000 cpm total for 16 hours, followed by a wash in 2 X SSC and 0.1% SDS solution, a wash of 4 times for 1 minute each at room temperature and 4 times at 60 degrees Celsius for 30 minutes each. Subsequent to drying, one exposes to film. For lower stringencies, the temperature of hybridization is reduced to about 12 degrees Celsius below the melting temperature (T_m) of the duplex. The T_m is known to be a function of the G-C content and duplex length as well as the ionic strength of the solution.

"Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. As noted above, the appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known

in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

As used herein, an "isolated nucleic acid fragment or sequence" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. (A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides, and most preferable at least about 25 nucleotides identical or complementary to a region of the specified nucleotide sequence.) Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

The terms "fragment or subfragment that is functionally equivalent" and "functionally equivalent fragment or subfragment" are used interchangeably herein. These terms

refer to a portion or subsequence of an isolated nucleic acid fragment in which the ability to alter gene expression or produce a certain phenotype is retained whether or not the fragment or subfragment encodes an active enzyme. For example, the fragment or subfragment can be used in the design of chimeric constructs to produce the desired phenotype in a transformed plant. Chimeric constructs can be designed for use in co-suppression or antisense by linking a nucleic acid fragment or subfragment thereof, whether or not it encodes an active enzyme, in the appropriate orientation relative to a plant promoter sequence.

The terms "homology", "homologous", "substantially similar" and "corresponding substantially" are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

"Native gene" refers to a gene as found in nature with its own regulatory sequences. In contrast, "chimeric construct" refers to a combination of nucleic acid fragments that are not normally found together in nature. Accordingly, a chimeric

construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that normally found in nature. (The term "isolated" means that the sequence is removed from its natural environment.)

A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric constructs. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" or "regulatory gene sequence" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. The sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence which can stimulate promoter or regulatory gene sequence activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoter sequences can also be located within the transcribed portions of genes, and/or downstream of the transcribed sequences. Promoters may be derived in their entirety from a native gene, or be composed

of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most host cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg, Biochemistry of Plants 15:1-82 (1989). It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity.

An "intron" is an intervening sequence in a gene that does not encode a portion of the protein sequence. Thus, such sequences are transcribed into RNA but are then excised and are not translated. The term is also used for the excised RNA sequences. An "exon" is a portion of the gene sequence that is transcribed and is found in the mature messenger RNA derived from the gene, but is not necessarily a part of the sequence that encodes the final gene product.

The "translation leader sequence" refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D. (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., Plant Cell 1:671-680 (1989).

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a DNA that is complementary to and synthesized from a mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro*. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Patent No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes. The terms "complement" and "reverse complement" are used

interchangeably herein with respect to mRNA transcripts, and are meant to define the antisense RNA of the message.

The term "endogenous RNA" refers to any RNA which is encoded by any nucleic acid sequence present in the genome of the host prior to transformation with the recombinant construct of the present invention, whether naturally-occurring or non-naturally occurring, i.e., introduced by recombinant means, mutagenesis, etc.

The term "non-naturally occurring" means artificial, not consistent with what is normally found in nature.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

The term "expression", as used herein, refers to the production of a functional end-product. Expression of a gene involves transcription of the gene and translation of the mRNA into a precursor or mature protein. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical

or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020).

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or pro-peptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and pro-peptides still present. Pre- and pro-peptides may be but are not limited to intracellular localization signals.

"Stable transformation" refers to the transfer of a nucleic acid fragment into a genome of a host organism, resulting in genetically stable inheritance. In contrast, "transient transformation" refers to the transfer of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without integration or stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. The term "transformation" as used herein refers to both stable transformation and transient transformation.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Sambrook").

The term "recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

"PCR" or "Polymerase Chain Reaction" is a technique for the synthesis of large quantities of specific DNA segments, consists of a series of repetitive cycles (Perkin Elmer Cetus

Instruments, Norwalk, CT). Typically, the double stranded DNA is heat denatured, the two primers complementary to the 3' boundaries of the target segment are annealed at low temperature and then extended at an intermediate temperature. One set of these three consecutive steps is referred to as a cycle.

Polymerase chain reaction ("PCR") is a powerful technique used to amplify DNA millions of fold, by repeated replication of a template, in a short period of time. (Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Erlich et al., European Patent Application No. 50,424; European Patent Application No. 84,796; European Patent Application No. 258,017; European Patent Application No. 237,362; Mullis, European Patent Application No. 201,184; Mullis et al., U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki et al., U.S. Patent No. 4,683,194). The process utilizes sets of specific in vitro synthesized oligonucleotides to prime DNA synthesis. The design of the primers is dependent upon the sequences of DNA that are to be analyzed. The technique is carried out through many cycles (usually 20-50) of melting the template at high temperature, allowing the primers to anneal to complementary sequences within the template and then replicating the template with DNA polymerase.

The products of PCR reactions are analyzed by separation in agarose gels followed by ethidium bromide staining and visualization with UV transillumination. Alternatively, radioactive dNTPs can be added to the PCR in order to incorporate label into the products. In this case the products of PCR are visualized by exposure of the gel to x-ray film. The added advantage of radiolabeling PCR products is that the levels of individual amplification products can be quantitated.

The terms "recombinant construct", "expression construct" and "recombinant expression construct" are used interchangeably herein. These terms refer to a functional unit of genetic material that can be inserted into the genome of a cell using standard methodology well known to one skilled in the art. Such construct may be itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon the method that will be used to transform host plants as is well known to those skilled in the art. For example, a plasmid can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising any of the isolated nucleic acid fragments of the invention. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) EMBO J. 4:2411-2418; De Almeida et al., (1989) Mol. Gen. Genetics *218*:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

A "monoclonal antibody" as used herein is intended to refer to one of a preparation of antibody molecules containing antibodies which share a common heavy chain and common light chain amino acid sequence, in contrast with an antibody from a "polyclonal" antibody preparation which contains a mixture of different antibodies. Monoclonal antibodies can be generated by several novel technologies like phage, bacteria, yeast or ribosomal display, as well as classical methods exemplified by hybridoma-derived antibodies (*e.g.*, an antibody secreted by a hybridoma prepared by hybridoma technology, such as the

standard Kohler and Milstein hybridoma methodology ((1975) Nature 256:495-497). Thus, a non-hybridoma-derived agonistic antibody of the invention is still referred to as a monoclonal antibody although it may have been derived by non-classical methodologies.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds to a globulomer is substantially free of antibodies that specifically bind antigens other than a globulomer). An isolated antibody that specifically binds a globulomer may, however, have cross-reactivity to other antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature*

341:544-546), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.*

(1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R., (1997) *TIB Tech.* 15:62-70; Azzazy H., and Highsmith W.E., (2002) *Clin. Biochem.* 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) *BioTechniques* 29:128-145; Hoogenboom H., and Chames P. (2000) *Immunology Today* 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L.L. (2002) *Current Opinion in Biotechnology* 13:593-597; Little M. et al (2000) *Immunology Today* 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that,

while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. (See also Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, 1991). The human antibodies of the present invention, however, may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). (See also Harlow and Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990).

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of V_H and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

Recombinant human antibodies of the present invention have variable regions, and may also include constant regions, derived from human germline immunoglobulin sequences. (See Kabat et al. (1991) *supra*.) In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis), and thus the amino acid sequences of the VH and VL regions of the

recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis or backmutation or both.

The term "backmutation" refers to a process in which some or all of the somatically mutated amino acids of a human antibody are replaced with the corresponding germline residues from a homologous germline antibody sequence. The heavy and light chain sequences of a human antibody of the invention are aligned separately with the germline sequences in the VBASE database to identify the sequences with the highest homology. VBASE is a comprehensive directory of all human germline variable region sequences compiled from published sequences, including current releases of GenBank and EMBL data libraries. The database has been developed at the MRC Centre for Protein Engineering (Cambridge, UK) as a depository of the sequenced human antibody genes (website: http://www.mrc-cpe.cam.ac.uk/vbase-intro.php?menu=901). Differences in the human antibody of the invention are returned to the germline sequence by mutating defined nucleotide positions encoding such different amino acids. The role of each amino acid thus identified as a candidate for backmutation should be investigated for a direct or indirect role in antigen binding, and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody. To minimize the number of amino acids subject to backmutation, those amino acid positions found to be different from the closest germline sequence, but identical to the corresponding amino acid in a second germline sequence, can remain, provided that the second germline sequence is identical and co-linear to the sequence

of the human antibody of the invention for at least 10, preferably 12, amino acids on both sides of the amino acid in question. Backmutation may occur at any stage of antibody optimization.

A "labeled binding protein" is a protein wherein an antibody or antibody portion of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

For purposes of the present invention, a "glycosylated binding protein" comprises a protein wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* **21** (2005), pp. 11-16). In contrast, glycosylation of the variable domain may

have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., et al., Mol. Immunol. (1993) 30:1361- 1367), or result in increased affinity for the antigen (Wallick, S.C., et al., Exp. Med. (1988) 168:1099-1109; Wright, A., et al., EMBO J. (1991) 10:2717 2723). Further, glycosylation site mutants can be made in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity but have increased or decreased binding activity are also contemplated.

Further, the glycosylation of the antibody or antigen-binding portion of the invention can modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such a glycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in International Application Publication No. WO 03/016466A2, and U.S. Patent Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, a modified antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc

structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. (See, for example, Shields, R.L. et al. (2002) J. Biol. Chem. 277:26733-26740; Umana et al. (1999) Nat. Biotech. 17:176-1, as well as, European Patent No: EP 1,176,195; International Application Publication Number WO 03/035835 and WO 99/5434280, each of which is incorporated herein by reference in its entirety.)

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a

mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S Patent Application Publication Nos. 20040018590 and 20020137134 and International Application Publication No. WO 05/100584 A2).

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant

glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. Preferably, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

The invention also provides a method for making the monoclonal antibodies of the invention from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human immunoglobulin loci. One may produce such animals using methods known in the art. In a preferred embodiment, the non-human animals may be rats, sheep, pigs, goats, cattle or horses. Antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. See, e.g., Harlow and Lane, *supra*. In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using an antigen (for example, a globulomer) or a portion thereof, or a cell expressing the antigen of interest. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in International Application Publication No. WO 00/37504, herein incorporated by reference.

The antibody-producing hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of

selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art. Preferably, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma derived from the same species as the non-human animal.

In one aspect, the invention provides hybridomas that produce monoclonal antibodies to be used in the treatment, diagnosis and prevention of Alzheimer's Disease. In a preferred embodiment, the hybridomas are mouse hybridomas. In another preferred embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an antibody against a globulomer.

Recombinant antibodies may be generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, International Application Publication No. WO 92/02551 and Babcock, J.S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848. In this method, single cells secreting antibodies of interest (*e.g.*, lymphocytes derived from the immunized animal) are screened using an antigen-specific hemolytic plaque assay, wherein the antigen (e.g., globulomer), or a fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for the antigen. Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (*e.g.*, human constant regions), in mammalian

host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to IL-18. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in International Application Publication No. WO 97/29131 and International Application Publication No. WO 00/56772.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a nonhuman species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody in which human CDR sequences are introduced into nonhuman VH and VL sequences to replace the corresponding nonhuman CDR sequences. In particular, the term "humanized antibody" is an antibody or a variant, derivative, analog or

fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In other embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG 1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may

be selected to optimize desired effector functions using techniques well-known in the art.

The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. Further, as used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional

structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the "on rate" constant for association of an antibody to the antigen to form the antibody/antigen complex as is known in the art.

The term "K_{off}", as used herein, is intended to refer to the "off rate" constant for dissociation of an antibody from the antibody/antigen complex as is known in the art.

The term "K_d", as used herein, is intended to refer to the "dissociation constant" of a particular antibody-antigen interaction as is known in the art.

The term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. Preferably, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties

that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho or ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine or lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase or alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains or epitope tags); and magnetic agents, such as gadolinium chelates.

The term "antibody conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. Preferably, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin, as well as analogs and homologs of these agents.

The terms "crystal", and "crystallized" as used herein, refer to an antibody, or antigen binding portion thereof, that

exists in the form of a crystal. Crystals are one form of the solid state of matter.

The term "immunize" refers herein to the process of presenting an antigen to an immune repertoire whether that repertoire exists in a natural genetically unaltered organism, or a transgenic organism modified to display an artificial human immune repertoire. Similarly, an "immunogenic preparation" is a formulation of antigen that contains adjuvants or other additives that would enhance the immunogenicity of the antigen. An example of this would be co-injection of a purified form of GLP-1 receptor with Freund's complete adjuvant into a mouse. "Hyperimmunization", as defined herein, is the act of serial, multiple presentations of an antigen in an immunogenic preparation to a host animal with the intention of developing a strong immune response.

One way of measuring the binding kinetics of an antibody is by surface plasmon resonance. The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the Biacore system (Biacore International, Upsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson *et al.* (1993) Annales de Biologie Clinique (Paris) 51:19-26; Jönsson *et al.* (1991) Biotechniques 11:620-627; Johnsson *et al.* (1995) Journal of Molecular Recognition 8:125-131; and Johnnson *et al.* (1991) Analytical Biochemistry 198:268-277.

A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of

pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable

for, for example, parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous,

intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be

appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating Alzheimer's Disease or related diseases or conditions. For example, one of the antibodies of

the subject invention or antibody portion thereof may be coformulated and/or coadministered with one or more additional antibodies that bind other targets.

In certain embodiments, a monoclonal antibody of the subject invention or fragment thereof may be linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989); Maliga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press (1995); Birren et al., Genome Analysis: Detecting Genes, 1, Cold Spring Harbor, New York (1998); Birren et al., Genome Analysis: Analyzing DNA, 2, Cold Spring Harbor, New York (1998); Plant Molecular Biology: A Laboratory Manual, eds. Clark, Springer, New York (1997)).

Uses of the Monoclonal Antibody

The monoclonal antibodies of the present invention (e.g., 8F5 and 8CF) have many interesting utilities. For example, the monoclonal antibodies may be used in the prevention, treatment and diagnosis of Alzheimer's Disease as described above. Further, the antibodies may be used in the development of anti-antibodies. Further, the hybridoma producing the

respective antibody allows for the steady production of a continuous source of identical monoclonal antibodies (i.e., reagents), thereby guaranteeing identity between antibodies in various experiments as well as therapeutic uses.

Also, the methods of the present invention allow one to prepare appropriate amounts of starting material for use in the preparation of further materials that, in turn, may be utilized in the production of monoclonal antibodies (or other antibodies) for the treatment of Alzheimer's Disease. As noted above, the antibodies may also be used for passive immunization in order to prevent Alzheimer's Disease or other related neurological conditions characterized by the same symptoms as Alzheimer's Disease such as cognitive impairment.

In one diagnostic embodiment of the present invention, an antibody of the present invention (e.g., 8F5), or a portion thereof, is coated on a solid phase (or is present in a liquid phase). The test or biological sample (e.g., whole blood, cerebrospinal fluid, serum, etc.) is then contacted with the solid phase. If antigen (e.g., globulomer) is present in the sample, such antigens bind to the antibodies on the solid phase and are then detected by either a direct or indirect method. The direct method comprises simply detecting presence of the complex itself and thus presence of the antigens. In the indirect method, a conjugate is added to the bound antigen. The conjugate comprises a second antibody, which binds to the bound antigen, attached to a signal-generating compound or label. Should the second antibody bind to the bound antigen, the signal-generating compound generates a measurable signal. Such signal then indicates presence of the antigen in the test sample.

Examples of solid phases used in diagnostic immunoassays are porous and non-porous materials, latex particles, magnetic particles, microparticles (see e.g., U.S. Patent No.

5,705,330), beads, membranes, microtiter wells and plastic tubes. The choice of solid phase material and method of labeling the antigen or antibody present in the conjugate, if desired, are determined based upon desired assay format performance characteristics.

As noted above, the conjugate (or indicator reagent) will comprise an antibody (or perhaps anti-antibody, depending upon the assay), attached to a signal-generating compound or label. This signal-generating compound or "label" is itself detectable or may be reacted with one or more additional compounds to generate a detectable product. Examples of signal-generating compounds include chromogens, radioisotopes (e.g., 125I, 131I, 32P, 3H, 35S and 14C), chemiluminescent compounds (e.g., acridinium), particles (visible or fluorescent), nucleic acids, complexing agents, or catalysts such as enzymes (e.g., alkaline phosphatase, acid phosphatase, horseradish peroxidase, beta-galactosidase and ribonuclease). In the case of enzyme use (e.g., alkaline phosphatase or horseradish peroxidase), addition of a chromo-, fluro-, or lumo-genic substrate results in generation of a detectable signal. Other detection systems such as time-resolved fluorescence, internal-reflection fluorescence, amplification (e.g., polymerase chain reaction) and Raman spectroscopy are also useful.

Examples of biological fluids which may be tested by the above immunoassays include plasma, whole blood, dried whole blood, serum, cerebrospinal fluid or aqueous or organo-aqueous extracts of tissues and cells.

The present invention also encompasses a method for detecting the presence of antibodies in a test sample. This method comprises the steps of: (a) contacting the test sample suspected of containing antibodies with anti-antibody specific for the antibodies in the patient sample under time and

conditions sufficient to allow the formation of anti-antibody/antibody complexes, wherein the anti-antibody is an antibody of the present invention which binds to an antibody in the patient sample; (b) adding a conjugate to the resulting anti-antibody/antibody complexes, the conjugate comprising an antigen (which binds to the anti-antibody) attached to a signal generating compound capable of detecting a detectable signal; and (d) detecting the presence of the antibodies which may be present in the test sample by detecting the signal generated by the signal generating compound. A control or calibrator may be used which comprises antibody to the anti-antibody.

The present invention also includes a vaccine comprising one of more of the antibodies described herein or a portion thereof and a pharmaceutically acceptable adjuvant (e.g., Freund's adjuvant or phosphate buffered saline).

Kits are also included within the scope of the present invention. More specifically, the present invention includes kits for determining the presence of antigens (e.g., globulomers) in a patient suspected of having Alzheimer's Disease or another condition characterized by cognitive impairment. In particular, a kit for determining the presence of antigens in a test sample comprises a) an antibody as defined herein or portion thereof; and b) a conjugate comprising a second antibody (having specificity for the antigen) attached to a signal generating compound capable of generating a detectable signal. The kit may also contain a control or calibrator which comprises a reagent which binds to the antigen as well as an instruction sheet detailing how the kit is to be utilized and the components of the kit.

The present invention also includes a kit for detecting antibodies in a test sample. The kit may comprise a) an anti-antibody specific (for example, one of the subject invention)

for the antibody of interest, and b) an antigen or portion thereof as defined above. A control or calibrator comprising a reagent which binds to the antigen may also be included. More specifically, the kit may comprise a) an anti-antibody (such as the one of the present invention) specific for the antibody and b) a conjugate comprising an antigen (e.g., globulomer) attached to a signal generating compound capable of generating a detectable signal. Again, the kit may also comprise a control of calibrator comprising a reagent which binds to the antigen and may also comprise an instruction sheet or package insert describing how the kit should be used and the components of the kit.

The kit may also comprise one container such as vial, bottles or strip, with each container with a pre-set solid phase, and other containers containing the respective conjugates. These kits may also contain vials or containers of other reagents needed for performing the assay, such as washing, processing and indicator reagents.

It should also be noted that the subject invention not only includes the full length antibodies described above but also portions or fragments thereof, for example, the Fab portion thereof. Additionally, the subject invention encompasses any antibody having the same properties of the present antibodies in terms of, for example, binding specificity, structure, etc.

Deposit Information: The hybridoma (ML5-8F5.1F2.2A2) which produces monoclonal antibody 8F5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on December 1, 2005 under the terms of the Budapest Treaty and was assigned ATCC No. PTA-7238.

Hybridoma (ML5-8C5.2C1.8E6.2D5) which produces monoclonal antibody 8C5 was deposited with the American Type Culture

Collection, 10801 University Boulevard, Manassas, Virginia 20110 on February 28, 2006 under the terms of the Budapest Treaty and was assigned ATCC No. PTA-7407.

The present invention may be illustrated by use of the following non-limiting examples:

EXAMPLE I(a)

PRODUCTION OF MONOCLONAL ANTIBODIES 8F5 AND 8C5

Balb/c mice were immunized sub-q with 50 microgram of A-beta (1-42)globulomer as described in Barghorn et al., 2005, J Neurochem, 95, 834-847 in CFA (Sigma) and boosted twice at one month intervals. Spleens were collected and spleen cells fused with mouse myeloma SP2/0 cells at 5:1 ratio by a PEG procedure. Fusion cells were plated in 96-well dishes in Azaserine/Hypoxanthine selection media at $2x10^5$ cells/ml, 200 ml per well. Cells were allowed to grow to form visible colonies and supernatants assayed for A-beta oligomer reactivity by a direct ELISA assay. Hybridomas secreting antibodies to A-beta oligomers were subcloned by limiting dilution, until antibody expression appeared stable.

EXAMPLE II

8F5 AND 8C5 PREFERENTIAL GLOBULOMER BINDING COMPARED TO MONOMER PREPARATIONS OF AB(1-40) AND AB(1-42)

To test the selectivity of 8F5, two differently dissolved Aß(1-42) monomer preparations were used as well as freshly prepared Aß(1-40) as surrogates for monomers. Two types of experiments were performed. In a first experiment, 8F5 was tested for Aß globulomer selectivity by a Sandwich-ELISA with globulomer derived but conformer non-specific MAb 6G1 (see S. Barghorn et al. J. Neurochemistry, 95:834 (2005)) as a capture

antibody. Biotinylated 8F5 was used as the second and conformer selective antibody. This experiment is described in Example 2.1 below.

In a second example, described in Example 2.2 below, the oligomer selectivity versus Aß(1-42) monomer and Aß(1-40) monomer was examined by dot blot immunoassay. In this experiment, 8F5 exhibited preferential binding to Aß(1-42) globulomer (compared to a known antibody 4G8 mapping to a similar region as 8F5, but derived from immunization with a linear peptide Aβ(17-24) (Abcam Ltd., Cambridge, MA)), as compared to Aß(1-42) monomer as well as compared to Aß(1-40) monomer. 8C5 was tested in an identical protocol to 8F5.

EXAMPLE 2.1: OLIGOMER SELECTIVITY OF MONOCLONAL ANTIBODY 8F5 AND 8C5

a) Preparation of Aß(1-42) globulomer:

9 mg Aß(1-42) Fa. Bachem were dissolved in 1.5ml HFIP (1.1.1.3.3.3 Hexafluor-2-propanol) and incubated 1,5 h at 37°C. The solution was evaporated in a SpeedVac and suspended in 396 µl DMSO (5mM Aß stock solution). The sample was sonified for 20 seconds in a sonic water bath, shaken for 10 minutes and stored over night at -20°C.

The sample was diluted with 4.5 ml PBS (20 mM NaH2PO4; 140 mM NaCl; pH 7,4) and 0.5 ml 2 % aqueous SDS-solution were added (0.2% SDS content). The mixture was incubated for 7 h at 37°C, diluted with 16 ml H_2O and further incubated for 16 hours at 37 deg C. After that, the Aß(1-42) globulomer solution was centrifuged for 20min at 3000g. The supernatant was concentrated to 0.5ml by 30KDa centriprep. The concentrate

was dialysed against 5mM NaH2PO4; 35mM NaCl; pH7.4 overnight at 6°C. Subsequently, the Aß(1-42) globulomer concentrate was centrifuged for 10min at 10000g. The supernatant was then aliquoted and stored at -20°C.

b) Preparation of monomer Aß(1-42), HFIP pretreated:

3mg human Aß(1-42),(Bachem Inc) cat. no. H-1368 were dissolved in 0.5ml HFIP (6mg/ml suspension) in an 1.7 ml Eppendorff tube and was shaken (Eppendorff Thermo mixer, 1400 rpm) for 1.5h at 37°C till a clear solution was obtained. The sample was dried in a speed vac concentrator (1.5h) and resuspended in 13.2µl DMSO, shook for 10 sec., followed by ultrasound bath sonification (20 sec) and shaking (e.g. in Eppendorff Thermo mixer, 1400 rpm) for 10 min.

6ml 20mM NaH2PO4; 140mM NaCl; 0.1% Pluronic F68; pH 7.4 was added and stirred for 1h at room temperature. The sample was centrifuged for 20min at 3000g. The supernatant was discarded and the precipitate solved in 0.6ml 20mM NaH2PO4; 140mM NaCl; 1% Pluronic F68; pH 7.4. 3.4ml water was added and stirred for 1h at room temperature followed by 20 min centrifugation at 3000 g. 8 x 0.5ml aliquots of the supernatant were stored at -20°.

c) Preparation of monomer Aß(1-42) in NH_4OH :

1mg Aß(1-42) solid powder (Bachem Inc. cat. no. H-1368) was dissolved in 0.5ml 0.1% NH_4OH in water (freshly prepared)(2mg/ml) and immediately shaken for 30 sec. at room temperature to get a clear solution. The sample was stored at -20°C for further use.

d) Preparation of monomer Aß(1-40):

1mg human Aß(1-40), (Bachem Inc) cat. no. H-1194 was suspended in 0.25ml HFIP (4mg/ml suspension) in an Eppendorff tube. The tube was shaken (e.g., in an Eppendorff Thermo mixer, 1400 rpm) for 1.5h at 37°C to get a clear solution and afterwards dried in a speed vac concentrator(1.5h). The sample was redissolved in 46µl DMSO (21.7 mg/ml solution), shaken for 10 sec., followed by 20 sec. sonification in ultrasound bath. After 10 min of shaking (e.g. in Eppendorff Thermo mixer, 1400 rpm), the sample was stored at -20°C for further use.

e) Biotinylation of anti-Aß mouse Mab 8F5:

500µl anti-Aß mouse Mab 8F5 (0.64mg/ml) in PBS were added to 2µl 20mg/ml Sulfo-NHS-Biotin (Pierce Inc. cat.no. 21420) freshly dissolved in water and shaken (e.g. in Eppendorff Thermo mixer, 1400 rpm), for 30 min, dialyzed 16h at 6°C in a dialysis tube against 500ml 20mM Na Pi; 140mM NaCl; pH 7.4. The dialysate was stored at -20°C for further use. 8C5 was biotinylated accordingly.

f) Sandwich-ELISA for Aß-samples:

g) Reagent List:

1. F96 Cert. Maxisorp NUNC-Immuno Plate Cat.No.: 439454
2. Binding antibody:
   Anti-Aß mouse MAb 6G1, solved in PBS; conc.: 0.4mg/ml; store at -20°C
3. Coating-Buffer:
   100mM Sodiumhydrogencarbonate; pH 9.6

4. Blocking Reagent for ELISA; Roche Diagnostics GmbH Cat. No.: 1112589
5. PBST-Buffer: 20mM NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4
6. Albumin bovine fraction V, protease-free; Serva Cat.No.: 11926.03; store at 4°C.
7. PBST + 0.5% BSA-Buffer: 20mM NaH2PO4; 140mM NaCl ; 0.05% Tween 20; pH 7.4 + 0.5% BSA
8. Aß(1-42)-globulomer Standard Stock: solution in 5mM NaH2PO4 ; 35mM NaCl; pH7.4; conc.: 10.77mg/ml; store at -20°C
9. Aß(1-42) monomer HFIP treated Standard Stock: solution in 3mM NaH2PO4; 21mM NaCl; 0.15% Pluronic F68; pH 7.4; conc.: 0.45mg/ml; store at -20°C
10. Aß(1-42) monomer in NH4OH Standard Stock; solution in 0.1% NH_4OH conc.: 2mg/ml; store at -20°C
11. Aß(1-40) monomer HFIP treated Standard Stock; solution in DMSO; conc.: 21.7mg/ml; store at -20°C
12. biotinylated anti-Aß mouse mAb clone 8F5; solution in PBS; conc.: 0.24mg/ml; store at -80°C
13. Streptavidin-POD conjugate; Fa.Roche Cat. No.: 1089153
14. staining: TMB; Roche Diagnostics GmbH Cat.No.: 92817060; 42mM in DMSO; 3% H_2O_2 in water; 100mM sodium acetate pH 4.9
15. Stop staining by adding 2M Sulfonic Acid solution

Preparation of reagents:

The following protocol was utilized:

1. Binding antibody

Thaw mMAb 6G1 stock solution and dilute 1:400 in coating buffer.

2. Blocking reagent:

Dissolve blocking reagent in 100ml water to prepare the blocking stock solution and store aliquots of 10ml at -20°C. Dilute 3ml blocking stock solution with 27ml water for each plate to block.

3. Aß Standard solutions:

a) Aß(1-42)-globulomer

- Add 1µl Aß(1-42)-globulomer standard stock solution to 1076µl PBST + 0.5% BSA = 10µg/ml
- Add 50µl 10µg/ml Aß(1-42)-globulomer standard solution to 4950µl PBST + 0.5% BSA = 100ng/ml

b) Aß(1-42) monomer HFIP-treated

- Add 10µl Aß(1-42) monomer HFIP-pretreated standard stock solution to 440µl PBST + 0.5% BSA = 10µg/ml
- Add 50µl 10µg/ml Aß(1-42) monomer HFIP pretreated standard solution to 4950µl PBST + 0.5% BSA = 100ng/ml

c) Aß(1-42) monomer in NH4OH

- Add 5µl Aß(1-42) monomer in NH4OH standard stock solution to 995µl PBST + 0.5% BSA = 10µg/ml
- Add 50µl 10µg/ml Aß(1-42) monomer in NH4OH standard solution to 4950µl PBST + 0.5% BSA = 100ng/ml

d) Aß(1-40) monomer HFIP-pretreated

- Add 1µl Aß(1-40) monomer HFIP pretreated standard stock solution to 49µl PBST + 0.5% BSA = 430µg/ml

- Add 10µl 430µg/ml Aß(1-40) monomer HFIP pretreated standard solution to 420µl PBST + 0.5% BSA = 10µg/ml
- Add 50µl 10µg/ml Aß(1-40) monomer HFIP pretreated standard solution to 4950µl PBST + 0.5% BSA = 100ng/ml

Standard curves:

No	Stock	PBST + 0.5% BSA	Final Conc.
1	2ml S	0 ml	100ng/ml
2	0.633ml (1)	1.367ml	31.6ng/ml
3	0.633ml (2)	1.367ml	10ng/ml
4	0.633ml (3)	1.367ml	3.16ng/ml
5	0.633ml (4)	1.367ml	1ng/ml
6	0.633ml (5)	1.367ml	0.32ng/ml
7	0.633ml (6)	1.367ml	0.1ng/ml
8	0ml	2ml	0.0ng/ml

1. Primary antibody: biotinylated mMAb 8F5:
The concentrated biotinylated anti-Aß mAb 8F5 was diluted in PBST + 0.5% BSA-buffer. The dilution factor was 1/1200 = 0.2µg/ml. The antibody was used immediately.

2. Label Reagent:
Reconstitute Streptavidin-POD conjugate lyophilizate in 0.5ml water. Add 500µl glycerol and store aliquots of 100µl at -20°C for further use.
Dilute the concentrated label reagent in PBST-Buffer. The dilution factor is 1/10000. Use immediately.

3. Staining Solution TMB:
Mix 20ml 100mM sodium acetate pH 4.9 with 200µl of the TMB solution and 29.5µl 3% peroxide solution. Use immediately.

Sample Plate Setup: (Note that all standards are run in duplicate)

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	100	100	100	100	100	100	100	100	100	100
B	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6
C	10	10	10	10	10	10	10	10	10	10	10	10
D	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16
E	1	1	1	1	1	1	1	1	1	1	1	1
F	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
G	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
H	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Procedure Utilized:

1. Apply 100µl anti-Aß mMAb 6G1 solution per well and incubate overnight at 4°C.

2. Discard the antibody solution and wash the wells with 250µl PBST-Buffer three times.

3. Add 260µl block solution per well and incubate 2h at room temperature.

4. Discard the block solution and wash the wells with 250µl PBST-Buffer three times.

5. After preparation of standards, apply 100µl per well of standards to the plate. Incubate 2h at room temperature and overnight at 4°C.

6. Discard the standard solution and wash the wells with 250µl PBST-Buffer three times.

7. Add 200µl primary biotinylated antibody 8F5 solution per well and incubate 1.5h at room temperature.

8. Discard the antibody solution and wash the wells with 250µl PBST-Buffer three times.

9. Add 200µl label solution per well and incubate 1h at room temperature.

10. Discard the label solution and wash the wells with 250µl PBST-Buffer three times.

11. Add 100µl of TMB solution to each well and incubate at room temperature (5-15min).

12. Observe staining and apply 50µl of the Stop Solution per well after beginning of background staining.

13. UV-read at 450nm.

14. Calculate results from standard curve.

15. Evaluation

The results are shown in Fig. 1 for the antibody 8F5 and in Fig. 8 for the antibody 8C5. Log EC50 values are significantly lowest for the Aß(1-42) globulomer antigen (1.958) compared to reduced values for two differently prepared Aß (1-42) monomers (2.745 and 3.003 respectively) and Aß(1-40) monomer (2.825). These data indicate about 10 fold

selectivity of antibody 8F5 for Aß (1-42) globulomer versus Aß (1-42) monomer.

Almost identical results were obtained with antibody 8C5 and are shown in Fig. 8.

EXAMPLE 2.2: OLIGOMER SELECTIVITY OF MONOCLONAL ANTIBODY 8F5 AND 8C5

-Discrimination of Aβ monomer against Aβ globulomer by dot blot method: Comparison of 8F5 and 8C5 versus 4G8.

Serial dilutions of Aβ(1-42) globulomer, Aβ1-42 monomer and Aβ1-40 monomer were made in the range from 100pmol/µl-0.01pmol/µl in PBS. Of each sample, 1µl was dotted onto a nitrocellulose membrane. The mouse monoclonal antibodies 4G8 and 8F5 (0.2 µg/ml) were used for detection with an anti-mouse IgG coupled to alkaline phosphatase as secondary antibody and the staining reagent NBT/BCIP (Roche Diagnostics, Mannheim). The detection signal was analyzed in its intensity (reflective density = RD) via a densitometer (GS 800, Biorad, Hercules, CA, USA) at an antigen concentration of 10pmol. At this concentration for every Aβ-form, the measured reflective density was in the linear range of the densitometer detection. The other antibody 8C5 was used in an analogous protocol. The results are shown in Table 1 below:

	Reflective Density (RD) [10pmol]				
	Aß(1-42) globulomer	Aß(1-42) monomer	Aß(1-40) monomer	Ratio RD Aß(1-42) globulomer / RD Aß(1-42) monomer	Ratio RD Aß(1-42) globulomer / RD Aß(1-40) monomer
8F5	1,6	1,1	0,1	1,4	16,9
8C5	1,3	0,2	0,3	5,1	4,1

4G8	3	3,1	0,7	1	4,2

Table 1: Discrimination of anti-Aβ-antibodies of Aβ1-40 monomer and Aβ1-42 monomer. The discrimination was calculated as the ratio of detection signal of Aβ1-42 globulomer and Aβ1-42 monomer, respectively Aβ1-40 monomer.

In particular, the above results indicate that 8F5_and 8C5 show a different binding profile compared to commercially available anti-Aβ(1-42) antibody to 4G8, which maps to Aβ (17-24)(i.e., a linear sequence). More specifically, 8F5 and 8C5 show a preference for globulomer binding versus Aβ42 monomer (see column 4; compare 1.4 versus 1) as well as a preference for globulomer binding versus Aβ40 (column 5; compare 16.9 versus 4.2). These two improved binding selectivities over standard 4G8 should result in the production of fewer side effects upon use of 8F5 and/or 8C5, as described above (e.g., plaque binding).

EXAMPLE III

BINDING OF 8F5 AND 8C5 TO Aß(1-42) FIBRILS

Since 8F5 antibody was generated against soluble globulomers, it was hypothesized that 8F5 should not bind to deposited plaque or fibril material. Therefore, binding of 8F5 to polymerized Aß fibril suspensions was tested as described in the following example:

- Preparation of Aß(1-42) fibrils:

1mg Aß(1-42) (Bachem Inc., Catalog Nr.: H-1368) was dissolved in 500µl aqueous 0.1% NH_4OH (Eppendorff tube), and the sample was stirred for 1min at room temperature followed by 5 min centrifugation at 10000 g. Supernatant was pipetted into a new Eppendorff tube and the Aß(1-42) concentration measured

according to Bradford protein concentration assay (BIO-RAD Inc. assay procedure).

100µl of this freshly prepared Aß(1-42) solution were neutralized with 300µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 followed by 2% HCl to adjust pH 7.4. The sample was incubated for another 20 hrs at 37°C and centrifuged (10min, 10000g). The supernatant was discarded and the fibril pellet resuspended with 400µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 under 1 min stirring on a Vortex mixer followed by centrifugation (10min, 10000g). After discarding the supernatant, this resuspending procedure was repeated, and the final fibril suspension spun down by another centrifugation (10min, 10000g). The supernatant was once again discarded and the final pellet resuspended in 380µl 20mM NaH2PO4; 140mM NaCl; pH7.4 under 1 min stirring on a Vortex mixer. Aliquots of the sample were stored at -20°C in a freezer.

80µl fibril suspension were mixed with 320µl 20mM NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4, buffer and stirred for 5min at room temperature followed by sonification (20 sec). After centrifugation (10min, 10000g), the pellet was resuspended with 190µl 20mM NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4 under stirring in a Vortex mixer.

- Binding of Antibodies to Aß(1-42) fibrils

10µl aliquots of this fibril suspension was incubated with:

a) 10µl 20mM Na Pi; 140mM NaCl; pH 7.4
b) 10µl 0.1µg/µl mMAb 6E10 Signet Inc. Cat.#9320 in 20mM
c) NaH2PO4; 140mM NaCl; pH 7.4
d) 10µl 0.1µg/µl mMAb 4G8 SignetInc. Cat# 9220 in 20mM Na Pi; 140mM NaCl; pH 7.4

e) 10µl 0.1µg/µl mMAb 8F5 (8C5) in 20mM Na Pi; 140mM NaCl; pH 7.4

Samples were incubated for 20h at 37°C. Finally the samples were centrifuged (10 min at 10000g). The supernatants containing the unbound antibody fraction were collected and mixed with 20 µl SDS-PAGE sample buffer. The pellet fractions were washed with 50µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 buffer under 1 min stirring in a Vortex mixer followed by centrifugation (10min, 10000g). The final pellets were resuspended in 20µl 20mM Na Pi; 140mM NaCl; 0.025% Tween 20; pH 7.4 buffer and solved in 20µl SDS-PAGE buffer.

-SDS-PAGE analysis

Supernatants and resuspended pellet samples were heated for 5 min at 98°C and loaded onto a 4-20% Tris/Glycin Gel under the following conditions:

SDS-sample buffer: 0.3g SDS; 0.77g DTT; 4ml 1M Tris/HCl pH 6.8; 8ml glycerol; 1ml 1% Bromphenolblue in Ethanol; add water to 50 ml 4-20% Tris/Glycin Gel:Invitrogen Inc., No.: EC6025BOX

running buffer: 7.5g Tris; 36g Glycine; 2.5g SDS; add water to 2.5l

The PAGE was run at 20 mA. Gels were stained by Coomassie Blue R250.

Results:

Coomassie staining of SDS-PAGE indicated the presence of heavy and light chains of antibodies predominantly in the

supernatant of the fibril suspension (lane 7, Figure 2), the remaining fibril suspension showed very little antibody material while also showing partly depolymerized Abeta at 4.5 kDa. In contrast to 8F5 and 8C5, other anti-Aß antibodies did not show up in the soluble fraction (6E10, lane 3, Figure 2) or only partly (4G8, lane 5, Figure 2) compared to fibril bound fraction (lane 6, Figure 2).

The relative binding to fibril type Abeta was evaluated from SDS-PAGE analysis by measuring the Reflective Density values from the heavy chain of the antibodies in the fibril bound and the supernatant fractions and calculated according to the following formula:

Fibril bound Ab fraction = $RD_{\text{fibril faction}}$ x100%/($RD_{\text{fibril faction}}$ + RD $_{\text{supernatant fraction}}$).

The following values were obtained:

antibody	Fibril bound Ab fraction
6E10	98%
8F5	16%
8C5	21%

These data indicate a significant reduction of bound 8F5 and 8C5 compared to standard antibody 6E10.

EXAMPLE IV

PREFERENTIAL BINDING OF ENDOGENOUS Aß(1-42) GLOBULOMERS COMPARED TO Aß(1-40)

Based upon the oligomer concept of Aß, it is important that anti-Aß oligomer antibodies also can demonstrate

preferential binding for Aß (1-42) oligomers *in vivo*, in particular, over Aß(1-40) in Mild Cognitive Impairment and AD patients. The concept of lowering Aß(1-42) species over Aß(1-40) is used in a therapeutic approach for the treatment of AD *via* NSAIDs (Weggen et al., *Nature* 414, 212-216 (2001)). It is assumed that those NSAIDs which lower Aß(1-42) in relation to Aß(1-40) display the best efficacy in the treatment of Alzheimer Disease. The Aß(1-42)/Aß(1-40) ratio is important for a selective therapy as well as for diagnostic purposes.

An analysis was performed with CSF samples from Alzheimer's Disease patients and patients with MCI. From the results shown in Figure 3 and described below, it can be concluded that 8F5 has a major advantage over Aß antibodies like 6E10 because 8F5 detects a higher ratio of Aß(1-42) over less aggregating Aß(1-40). This advantage will allow one to more selectively diagnose and neutralize Aß(1-42) type oligomers in MCI and AD patients.

A) ENDOGENOUS AMYLOID ß(1-42) AND AMYLOID ß(1-40) LEVELS IN CSF OF MCI AND AD PATIENTS AFTER IMMUNOPRECIPITATION WITH OLIGOMER SELECTIVE ANTI-Aß MURINE MAB 8F5:

Immobilization of anti-Aß mMAB`s to CNBr-activated Sepharose 4B:

a) mMAb 6E10 Signet Inc., Cat.no. 9320

b) mMAb 8F5

0.4g CNBr-activated Sepharose 4B (Amersham Pharmacia Bio-tech AB, Uppsala, Sweden, Inc., No.: 17-0430-01) were added to 10ml aqueous 1mM HCl and incubated for 30min at room temperature. The CNBr-activated Sepharose 4B was washed three times with 10ml 1mM HCl and twice with 10ml 100mM $NaHCO_3$; 500mM NaCl; pH 8.3. For each of the immobilized antibodies, 100µl CNBr-activated Sepharose 4B Matrix were added to 950µl 0.5mg/ml

anti-Aß mMAb solution in 100mM $NaHCO_3$; 500mM NaCl; pH 8.3. After 2 h of shaking at room temperature, samples were centrifuged for 5min at 10000g. Then, 500µl 100mM Ethanolamine; 100mM $NaHCO_3$; 500mM NaCl; pH 8.3, buffer was added to the beads, and samples were shaken for 1h at room temperature. The anti-Aß mMAb-Sepharose samples were centrifuged for 5min at 10000g and washed 5 times with 500µl 20mM NaH2PO4; 140mM NaCl; pH 7.4. Before storage at 6°C, samples were stabilized by adding sodium azide to 0.02% final concentration.

Immunoprecipitation:

a) mMAb 6E10-Sepharose
b) mMAb 8F5 -Sepharose

200µl of the human Cerebral Spinal Fluid samples were diluted with 200µl 20mM NaH2PO4NaH_2PO_4; 140mM NaCl; 0.05% Tween 20; pH 7.4. These samples were added to 2µl anti-Aß mMAb-Sepharose Matrix and stirred for 2h at room temperature. The samples were centrifuged for 5min at 10000g. The supernatants were discarded and the anti-Aß mMAb-Sepharose washed twice with 50µl PBS, stirred for 1min and centrifuged (5min at 10000g). The supernatants were discarded, and the Sepharose beads were now suspended in 50µl 2mM NaH_2PO_4NaH2PO4; 14mM NaCl, pH7.4, followed by 1min of stirring at room temperature and 5min of centrifugation at 10000g. In a next step, the anti-Aß mMAb-Sepharose beads were treated with 50µl 50% CH_3CN; 0.2% TFA in water. After 10min shaking at room temperature, samples were centrifuged 5min at 10000g. The supernatants were collected and transferred to 1.5ml Eppendorf tubes. Samples were mixed with 50µl water and evaporated in a Speed Vac concentrator.

The pellet was redissolved in 4µl 70% HCOOH, shaken for 10min at room temperature and neutralized with 76µl 1M Tris-solution and 720µl 20mM NaH_2P0_4NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4.

Samples for the Determination of Aß(1-40); (1-42) Monomer Forms in CSF:

a) Aß-content in CSF-samples without immunoprecipitation:

158µl CSF were diluted with 342µl 20mM NaH_2P0_4; 140mM NaCl; 0.05% Tween 20; pH 7.4. This 1:3.16 dilution was taken for Sandwich ELISA´s and taken into account during evaluation.

b) Aß-content in CSF-samples after immunoprecipitation:

Samples from the above-mentioned procedure were taken for analysis.

Sandwich-ELISA Protocol Used for the Determination of Aß(1-40) in CSF

Reagent List:

1. F96 Cert. Maxisorp NUNC-Immuno Plate Cat.No.: 439454
2. Binding antibody
   Anti-Aß mAb clone 6E10; Signet Cat.No. 9320; conc.: 0.4mg/ml Bradford (BioRad); store at -20°C
3. Coupling-buffer
   100mM sodiumhydrogencarbonate; pH9.6

4. Blocking Reagent for ELISA; Roche Diagnostics GmbH
   Cat.No.: 1112589
5. PBST-buffer
   20mM $NaH_2P0_4NaH2PO4$; 140mM NaCl; 0.05% Tween 20; pH7.4
6. Aß(1-40) Standard:
   Aß(1-40) solid powder; Bachem Cat.No.: H-1194; store at -20°C
7. Primary antibody:
   anti-Aß (1-40) rabbit pAb; affinity purified; solution in PBS; conc.: 0.039mg/ml ;
   Signet Cat.No. 9130-005; store at -20°C
8. Label reagent:
   anti-rabbit-POD conjugate; Fa.Jackson ImmunoResearch
   Cat.No.: 111-036-045;
9. Staining:
   TMB; Roche Diagnostics GmbH Cat.No.: 92817060; 42mM in DMSO; 3% H_2O_2 in water; 100mM sodium acetate
   pH 4.9
10. Stop Solution 2M Sulfonic Acid

Protocols Used For Preparation of Reagents:

1. Binding antibody:
   anti-Aß mAb 6E10 (Signet Inc, Catalog # 9320) is diluted to a final concentration of 0.7 microg/ml.

2. Blocking reagent:
   For preparation of the blocking stock solution the blocking reagent is dissolved in 100 ml H_2O and stored at -20 °C in aliquots of 10 ml each. 3 ml of the blocking stock solution are diluted with 27 ml H_2O for blocking one ELISA plate.

3. Aß(1-40) monomer form standard dilution:

A) Aß(1-40) monomer Standard Stock: dissolve 0.5mg Aß(1-40) in 250µl 0.1%NH_4OH, conc.: 2mg/ml ; freshly prepared; use immediately.

B) Add 5µl Aß(1-40)-monomer standard stock solution to 995µl PBST = 10µg/ml

C) Add 5µl, 10µg/ml Aß(1-40)-monomer standard solution to 4995µl PBST = 10ng/ml

Standard curve:

No Final conc.	Stock	PBST
1 10000pg/ml	2ml B	0ml
2 3160pg/ml	0.633ml (1)	1.367ml
3 1000pg/ml	0.633ml (2)	1.367ml
4 316pg/ml	0.633ml (3)	1.367ml
5 100pg/ml	0.633ml (4)	1.367ml
6 31.6pg/ml	0.633ml (5)	1.367ml
7 10pg/ml	0.633ml (6)	1.367ml
8 0.0pg/ml	0ml	2ml

Samples:

IP : immunoprecipitate samples

No dilution factor	sample	PBST
1 directly	0.4ml IP	0ml
2 1:5	0.1ml (1)	0.4ml
3 1:25	0.1ml (2)	0.4ml
4 1:125	0.1ml (3)	0.4ml

4. Primary antibody:
Dilute the concentrated anti-Aß (1-40) pAb in PBST buffer. The dilution factor is 1/200 = 0.2µg/ml. Use immediately.

5. Secondary antibody:
Lyophilized anti-rabbit-POD conjugate is dissolved in 0.5 ml H_2O and mixed with 500 µl glycerol. The antibody concentrate is then stored at -20 °C in aliquots of 100 µl. The concentrate is diluted 1:10'000 in PBST buffer. The antibody solution is used immediately.

6. TMB solution:
20 ml of 100 mM sodium acetate, pH 4.9, are mixed with 200 µl TMB solution and 29.5 µl of 3% hydrogen peroxide. This solution is used immediately.

Sample Plate Setup: (Note that all standards and samples are run in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	10000	10000	U1	U1								
B	3160	3160	U2	U2								

C	1000	1000	U3	U3								
D	316	316	U4	U4								
E	100	100	U5	U5								
F	31.6	31.6	U6	U6								
G	10	10	U7	U7								
H	0.0	0.0	U8	U8								

U1-U# = Unknown samples

Procedure Used:

1. Apply 100µl binding antibody solution per well and incubate overnight at 4°C.
2. Discard the antibody solution and wash the wells with 250µl PBST-buffer for three times.
3. Add 260µl block solution per well and incubate 2h at room temperature.
4. Discard the block solution and wash the wells with 250µl PBST-buffer for three times.
5. After preparation of standards and samples, apply 100µl per well of standards and samples to the plate and incubate 2h at room temperature and overnight at 4°C.
6. Discard the standard/sample solution and wash the wells with 250µl PBST-buffer for three times.
7. Add 200µl primary antibody solution per well and incubate 1.5h at room temperature.
8. Discard the antibody solution and wash the wells with 250µl PBST-buffer for three times.
9. Add 200µl label solution per well and incubate 1h at room temperature.
10. Discard the label solution and wash the wells with

250µl PBST-buffer for three times.

11. Add 100µl of TMB solution to each well and incubate at room temperature (5-15min).
12. Observe colour development and apply 50µl of the Stop solution per well.
13. Read at 450nm.
14. Calculate results from standard curve.
15. Evaluation:

If extinction from unknown samples is not in the linearity range of the calibration curve, repeat ELISA with appropriated sample dilution.

Sandwich-ELISA Protocol Used for the Determination of Aß(1-42) Monomer Form in CSF

Reagent List:

1. F96 Cert. Maxisorp NUNC-Immuno Plate Cat.No.:439454
2. Binding antibody
   Anti-Aß mAb clone 6E10 ; Signet Cat.No. 9320 ; conc.: 0.4mg/ml Bradford (BioRad) ; store at -20°C
3. Coating-Buffer
   100mM sodiumhydrogencarbonate; pH9.6
4. Blocking Reagent for ELISA; Roche Diagnostics GmbH
   Cat.No.: 1112589
5. PBST-Buffer
   20mM NaH2PO4NaH2PO4; 140mM NaCl; 0.05% Tween 20 ; pH7.4
6. Aß(1-42) Standard:
   Aß(1-42) solid powder; Bachem Cat.No.: H-1368; store at -20°C
7. Primary antibody:

anti-Aß (1-42) rabbit pAb; affinity purified; biotinylated ; solution in PBS with 50% glycerol; conc.: 0.25mg/ml; Signet Cat.No. 9137-005; store at -20°C

8. Label reagent:
anti-rabbit-POD conjugate; Fa. Jackson ImmunoResearch Cat.No.: 111-036-045

9. Staining:
TMB; Roche Diagnostics GmbH Cat. No.: 92817060;
42mM in DMSO
3% H_2O_2 in water
100mM sodium acetate, pH4.9
Stop Solution: 2M Sulfonic Acid

Method Used In Preparation of Reagents:

1. Binding antibody:
Dilute anti-Aß mAb clone 6E10 1:400 in coating buffer.

2. Blocking reagent:
Dissolve blocking reagent in 100ml water to prepare the blocking stock solution and store aliquots of 10ml at -20°C. Dilute 3ml blocking stock solution with 27ml water for each plate to block.

3. Aß(1-42) monomer form, standard dilution:
Aß(1-42) Monomer Standard Stock: dissolve 0.5mg Aß(1-42) in 250µl 0.1%NH_4OH; conc.: 2mg/ml; freshly prepared; use immediately.
Add 5µl Aß(1-42)-monomer standard stock solution to 995µl PBST = 10µg/ml.
Add 5µl, 10µg/ml Aß(1-42)-monomer standard solution to 4995µl PBST = 10ng/ml.

Standard curve:

No Final conc.	Stock	PBST
1 10000pg/ml	2ml B	0ml
2 3160pg/ml	0.633ml (1)	1.367ml
3 1000pg/ml	0.633ml (2)	1.367ml
4 316pg/ml	0.633ml (3)	1.367ml
5 100pg/ml	0.633ml (4)	1.367ml
6 31.6pg/ml	0.633ml (5)	1.367ml
7 10pg/ml	0.633ml (6)	1.367ml
8 0.0pg/ml	0ml	2ml

Samples:

IP : immunoprecipitate samples

No dilution factor	sample	PBST
1 directly	0.4ml IP	0ml
2 1:5	0.1ml (1)	0.4ml
3 1:25	0.1ml (2)	0.4ml
4 1:125	0.1ml (3)	0.4ml

Procedure Used:

1. Primary antibody:
   Dilute the concentrated anti-Aß (1-42) pAb in PBST buffer. The dilution factor is 1/1250 = 0.2µg/ml. Use immediately.

2. Label Reagent:
   Reconstitute anti-rabbit-POD conjugate lyophilizate in 0.5ml water. Add 500µl glycerol and store aliquots of 100µl at -20°C for further use.
   Dilute the concentrated Label reagent in PBST-buffer. The dilution factor is 1/5000. Use immediately.

3. TMB solution:
   Mix 20ml 100mM sodium acetate pH4.9 with 200µl of the TMB solution and 29.5µl 3% Peroxide solution. Use immediately.

Sample Plate Setup: (Note that all standards and samples are run in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	10000	10000	U1	U1								
B	3160	3160	U2	U2								
C	1000	1000	U3	U3								
D	316	316	U4	U4								
E	100	100	U5	U5								
F	31.6	31.6	U6	U6								
G	10	10	U7	U7								
H	0.0	0.0	U8	U8								

U1-U# = Unknown samples

Procedure Used:

1. Apply 100µl binding antibody solution per well and incubate overnight at 4°C.
2. Discard the antibody solution and wash the wells with 250µl PBST-buffer for three times.
3. Add 260µl block solution per well and incubate 2h at room temperature.
4. Discard the block solution and wash the wells with 250µl PBST-buffer for three times.
5. After preparation of standards and samples, apply 100µl per well of standards and samples to the plate. Incubate 2h at room temperature and overnight at 4°C.
6. Discard the standard/sample solution and wash the wells with 250µl PBST-buffer for three times.
7. Add 200µl primary antibody solution per well and incubate 1.5h at room temperature.
8. Discard the antibody solution and wash the wells with 250µl PBST-buffer for three times.
9. Add 200µl label solution per well and incubate 1h at room temperature.
10. Discard the label solution and wash the wells with 250µl PBST-buffer for three times.
11. Add 100µl of TMB solution to each well and incubate at room temperature (5-15min).
12. Observe color staining and apply 50µl of the Stop Solution per well.
13. Read at 450nm.
14. Calculate results from standard curve.
15. Evaluation:

If extinction from unknown samples is not in the linearity range of the calibration curve, repeat ELISA with appropriate sample dilution.

RESULTS:

	Aß40 ELISA (Signet)		Aß42 ELISA (Signet)		Aß42/40
MCI samples (n=4)	Aß(1-40)	SEM	Aß(1-42)	SEM	
without IP	11678,9	2879,4	1242,0	353,5	7,84%
6E10 IP	8282,4	2185,7	2035,1	280,9	17,35%
8F5 IP	8586,1	2396,8	2654,6	411,4	20,95%
AD samples (n=2)	Aß(1-40)	SEM	Aß(1-42)	SEM	
without IP	7297,5	1464,5	843,0	157,5	10,95%
6E10 IP	5610,2	28,3	1453,0	14,5	20,57%
8F5 IP	4133,9	86,9	1670,2	12,3	28,78%

The above results indicate the following:

a. A globulomer preferential antibody like 8F5 (or 8C5), in comparison to a non-globulomer selective antibody like 6E10, binds preferentially to Aβ42 compared to Aβ40 independent from the disease state. This result is indicative of a successful treatment for Alzheimer's Disease because preferentially eliminating Aβ42 over Aβ40 is being followed as a concept in AD-treatment, e.g., by the use of R-flubiprofen, Flurizan which has demonstrated efficacy in AD treatment in a clinical trial published by Myriad Inc. This concept was published by S. Weggen et al. (J Biol Chem. (2003) 278(34):31831-7). The results are shown in Figure 3.

b. A globulomer preferential antibody like 8F5 (or 8C5) binds to even more Aβ42 than Aβ40 in patients compared to healthy controls. This result is even more indicative of a successful treatment for Alzheimer's Disease because, as noted above, preferentially eliminating Aβ42 over Aβ40 is being followed as a concept in AD-treatment (e.g., by the use of

non-steroidal anti-inflammatory drugs, like R-flubiprofen). (See Figure 3.)

B) ENDOGENOUS AMYLOID ß(1-42) AND AMYLOID ß(1-40) LEVELS IN HUMAN CSF AFTER IMMUNOPRECIPITATION WITH GLOBULOMER SELECTIVE ANTI-Aß MURINE MAB 8F5 OR 8C5 IN COMPARISON WITH GLOBULOMER UNSELECTIVE ANTIBODY 6E10:

b1) Immunoprecipitation (IP) with Dynabeads M-280 Sheep anti-Mouse IgG

Abeta-antibody solutions

The following pure antibodies were obtained from hybridomas according to standard purification procedures:

- mMab 6E10; Fa.Signet Nr.: 9320; 1mg/ml in PBS buffer
- mMab 8F5; 1.65mg/ml in PBS buffer
- mMab 8C5; 1.44mg/ml in PBS buffer

Dynabeads M-280 Sheep anti-Mouse IgG:

Sheep anti-Mouse IgG (Invitrogen Inc., Cat. no.: 112.02) is covalently bound to magnetic beads (Dynabeads).

Activation of Dynabeads with monoclonal mouse antibodies

- The stock-suspension of dynabeads (Dynabeads M-280 Sheep anti-Mouse IgG, Invitrogen; Prod. No. 112.02) was shaken carefully to prevent foaming.
- 1 mL was aseptically removed and transferred to a 1.5 mL reaction vial.
- The dynabeads were washed 3 times 5 min with 1 mL immunoprecipitation (IP)-wash buffer (IP-wash-buffer: PBS

(20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1% (w/v) BSA). During the washing procedure, the supernatant was carefully removed while the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS).

- The washed dynabeads were incubated with 40 µg Abeta-antibody in 1 mL PBS, 0.1% (w/v) BSA.
- The activation was carried out by overnight incubation under shaking at 4°C.
- The activated dynabeads were washed 4 times 30 min (again using the MSS) with 1 mL IP-wash buffer (PBS (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1% (w/v) BSA).
- The activated dynabeads were resuspended with 1 mL PBS, 0.1% (w/v) BSA, 0.02 (w/v) % Na-Azide; vortexed and centrifuged briefly.
- The antibody activated dynabeads were stored at 4°C until further use.

CSF Sample preparation:

400 µL CSF from an Alzheimer's disease patient were added to 4 µL Complete Protease Inhibitor Cocktail (Roche Inc. Cat. no.: 1697498, 1 tablet dissolved in 1 mL water) and 0.8 µL 500 mM PMSF dissolved in methanol. After 10 min., 1.6 mL 20 mM NaH_2PO_4,140 mM NaCl, 0.05% Tween 20, pH 7.4 (PBST) was added.

Immunoprecipitation of Abeta species from human AD-CSF:

250 µL aliquot of the prepared CSF sample were added to 25 µL anti-Aß-Dynabeads suspension.

- Immunoprecipitation occurred under stirring at 6°C for 16 hours. Subsequent washing of the beads was performed 3 times 5 min. with 1 mL PBS/0,1% (w/v) BSA and finally once 3 min. with 1 mL 10 mM Tris/HCL pH 7.5 buffer. During the washing procedure, the supernatant was carefully removed while the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS).

The residual supernatant was thoroughly removed after the final washing step. The Abeta peptides and the corresponding antibody were removed from the Dynabeads by adding 25 µL sample buffer without β-Mercaptoethanol (0.36 M Bistris, 0.16 M Bicine, 1% SDS (w/v), 15% (w/v) sucrose, 0.004% (w/v) Bromphenolblue) to the Eppendorff tube and heating for 5 min at 95°C in a heating block. After cooling to room temperature, the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS), and the supernatant was transferred to another Eppendorff tube (IP eluate).

Analysis of Abeta immunoprecipitates by urea-PAGE followed by Western Blot procedure:

The quantification of Aß1-40 and Aß1-42 species was performed by a 8 M Urea Poly-Acrylamide-Gel-Electrophoresis system and subsequent Western Blot analysis according to the procedure first described by H.W. Klafki et al., Analytical Biochemistry 237, 24-29 (1996) and later also used by J. Wiltfang et al., J. of Neurochemistry 81, 481-496, 2002. There were only two minor changes made in the experimental procedure:

1) SDS concentration in the stacking gel was adjusted to 0.25% (w/v) instead of 0.1% (w/v).
2) For the Western blot the antibody 1E8 (Senetek Drug Delivery Technologies Inc. St.Louis, MO, USA) was replaced by Anti-Human Amyloid β (N) (82E1) Mouse IgG mAb (IBL, Cat.no.: 10323)

15 µL IP eluate aliquots of the immunoprecipitated samples were loaded onto the 8 M Urea PAGE. Electrophoresis was performed at 100 V (15 min) and continued at 60 V. The electrophoresis was stopped when the running front of the blue sample loading dye was still 0.5 cm away from the end of the gel.

Western blot procedure:

Western blot analysis was performed in a Semi Dry Blotting chamber (BioRad Inc., 45min at 75mA) onto 7.5cm x 9cm Nitrocellulose 0.45µm (BioRad Inc.).

Blotting buffer: 6 g Tris; 28.1 g Glycin; 500m L Methanol; adjust to 2.5 l with water.

The Nitrocellulose blot was boiled for 10 min in PBS at 100°C. The blot was saturated by treatment with 50 mL 5 % (w/v) BSA in PBST for 1 hour at RT. After removal of the fluid phase, the following washing step was performed twice with: 50 mL TTBS (25 mM Tris / HCl; 150 mM NaCl Puffer; 0.05% Tween 20; pH 7.5) for 10 min at RT and subsequently with 50 mL TBS (25 mM Tris / HCl; 150 mM NaCl buffer; pH 7.5) for 10 min at RT.

For further development, the final washing buffer was discarded from the blot and 15 mL antibody I solution (0.2 µg/mL 82E1 = 1:500 in 3 % (w/v) skimmed milk powder (Lasana Inc.), in 15 mL TBS) were added for 20 hours at 6°C. Removal of buffer was followed by the three wash steps as described above. The blot was incubated with Antibody solution II (1:10000 dilution of anti-Mouse -POD in 15 mL 3 % (w/v) skimmed milk powder in 15 mL TBS) for 1 hour at RT. Removal of buffer was followed by the three wash steps as described above.

After removal of the last washing buffer, 2 mL Super Signal West Femto Maximum Sensitivity Substrate Enhancer and 2 mL Peroxide Solution was mixed. The freshly prepared solution was poured onto the blot which was preincubated in the dark for 5 min. Chemiluminescence was recorded using a VersaDoc Imaging system(BioRad).

Imaging parameters:
- exposure time 180 sec.
Picture records after 30 sec., 60sec., 120 sec. and 180 sec.

The results were obtained from the picture with 180 sec. exposure time.

	Aß40 urea-PAGE [pg/ml]	Aß42 urea-PAGE [pg/ml]	Ratio Aß42/Aß40+42 x100%
6E10 IP	4389	202	4.4%
8F5 IP	1260	112	8.1%
8C5 IP	1202	211	14.9%

The above results indicate that a globulomer preferential antibody like 8F5 or 8C5, in comparison to a non-globulomer selective antibody like 6E10, binds to more Aβ42 than Aβ40 in human CSF. This result is indicative of a successful treatment for Alzheimer's Disease because, as noted above, preferentially eliminating Aβ42 over Aβ40 is being following as a concept in AD-treatment (e.g., by the use of R-flubiprofen (see above)).

EXAMPLE V

8F5 IMPROVES NOVEL OBJECT RECOGNITION IN APP TRANSGENIC MICE

In order to test a positive effect on cognition by neutralizing internal Aß(1-42) globulomer epitope with antibody 8F5, a passive immunization experiment with APP transgenic mice was performed in which the mice were tested for their ability to remember objects they have investigated before. After some time, delay between first and second encounter of objects, APP transgenic mice are not able to recognize the already investigated object. This experiment is based on the natural curiosity of the animals, and a significant lack of interest in the already investigated object demonstrates recognition of the object.

EXAMPLE V.1: INCREASED RECOGNITION INDEX BY MONOCLONAL ANTIBODY 8F5:

Animals:

Female mice of a single transgenic mouse model of Alzheimer's Disease in FVB x C57Bl background (APP/L, ReMYND, Leuven, Belgium) and negative litter mates as wild type controls in FVB x C57Bl background with an age of 3 months were used. All

mice were genotyped by polymerase chain reaction (PCR) at the age of 3 weeks and received a unique identity number, once the PCR results were known and were double checked by a second PCR before the onset of the study. All mice were randomized and age-matched, i.e., they were given a random number by computer and allocated randomly to a treatment. Animals were caged by treatment group 18 days before the onset of the study in order to allow them to familiarize to the new cage context. Mice had free access to pre-filtered and sterile water (UV-lamp) and standard mouse chow. The food was stored under dry and cool conditions in a well-ventilated storage room. The amount of water and food was checked daily, supplied when necessary and refreshed twice a week. Mice were housed under a reversed day-night rhythm: 14 hours light/10 hours darkness starting at 7 p.m. in standard metal cages type RVS T2 (area of 540 cm2). The cages are equipped with solid floors and a layer of bedding litter. The number of mice per cage was limited in accordance with legislation on animal welfare. Five days before the onset of the behavior test, mice were replaced in macrolon Type 2 cages and transported to the laboratory in order to adapt to the laboratory environment in preparation for the behavior test.

Treatment (Passive Immunization):

Three individual experiments were performed in which the mice (at least 9 per group) received intraperitoneal injections (500 μg in 240μL / mouse) at days 1, 8 and 15. Mice were treated with monoclonal antibodies 6G1, 8F5 and other non-disclosed antibodies, all dissolved in phosphate-buffered saline, or with 320 μL phosphate-buffered saline.

Novel object recognition test:

The novel object recognition test was performed on the day of the third treatment. The protocol used followed the method as described by Dewachter *et al.* (Journal of Neuroscience, 2002, 22(9):3445-3453). Mice were familiarized for one hour to a Plexiglas open-field box (52 x 52 x 40 cm) with black vertical walls and a translucent floor, dimly illuminated by a lamp placed underneath the box. The next day, the animals were placed in the same box and submitted to a 10 minute acquisition trial. During this trial, mice were placed individually in the open field in the presence of 2 identical objects A (orange barrel or green cube, similar size of ± 4 cm), and the duration ($time_{AA}$) and the frequency ($Freq_{AA}$) exploring object A (when the animals snout was directed towards the object at a distance of < 1 cm and the mice were actively sniffing in the direction of the object) was recorded by a computerized system (Ethovision, Noldus information Technology, Wageningen, Netherlands). During a 10 minute retention trial (second trial) performed 2.5 hours later, a novel object (object B, green cube or orange barrel) was placed together with the familiar object (object A) into the open field ($Freq_A$ and $Freq_B$ and $Time_A$ and $Time_B$, respectively). The recognition index (RI), defined as the ratio of the duration in which the novel object was explored over the duration in which both objects were explored [$Time_B$ / ($Time_A$ + $Time_B$) x 100], was used to measure non-spatial memory. The duration and frequency that object A was explored during the acquisition trial ($Time_{AA}$ and $Freq_{AA}$) was used to measure curiosity.

Analysis of data was done by combining APP transgenic mice that received monoclonal antibodies 6G1 or 8F5 or phosphate-buffered saline, and non-transgenic littermates that received phosphate-buffered saline, from all three studies (Fig. 4).

Mice that do not distinguish between an old object and a novel object have a recognition index of 50. Mice that recognize the old object will preferably explore the novel object and hence the recognition index becomes > 50. Mice that exclusively explore the novel object have a recognition index of 100. The mean recognition index per group was compared against chance level, i.e., 50, by t-test. The mean recognition index of all groups was also compared by ANOVA followed by a post-hoc t-test. The difference between PBS and wild type groups indicated a cognitive deficit of APP transgenic mice in this paradigm. PBS-injected mice performed at chance level (i.e., not significantly different from 50) while all other mice showed object recognition (Fig. 4: stars). When the performance of antibody-treated APP transgenic mice was compared with control groups, a significant difference was found versus PBS-treated but not versus wild-type mice (Fig. 4: circles) indicating that treatment with antibody 8F5 reversed the cognitive deficit in these APP transgenic mice.

EXAMPLE VI

IN SITU ANALYSIS OF THE SPECIFIC REACTION OF ANTIBODIES 8F5 AND 8C5 TO FIBRILLAR AMYLOID BETA PEPTIDE IN THE FORM OF AMYLOID PLAQUES AND AMYLOID IN MENINGEAL VESSELS IN OLD APP TRANSGENIC MICE AND ALZHEIMER'S DISEASE PATIENTS

Antibodies 8F5 and 8C5 show reduced staining to fibrillar Aβ peptide deposits suggesting that their therapeutic effect is mediated by binding to soluble globulomeric forms rather than fibrillar deposited forms of Aβ peptide. Since antibody binding to fibrillar Aβ peptide can lead to fast dissolution of aggregates and a subsequent increase of soluble Aβ concentration, which in turn is thought to be neurotoxic and

could lead to microhemorrhages, an antibody therapy that effects the soluble globulomer rather than the monomer is preferred.

Methods:

For these experiments, several brain material samples were used: cortical tissue from 2 AD patients (RZ16 and RZ 55)and cortical tissue from 19 month old Tg2576 mice (APPSWE # 001349,Taconic, Hudson, NY, USA) or 12 month old APP/L mice (ReMYND, Leuven, Belgium).
The mice overexpress human APP with a familial Alzheimer's disease mutation and form β-amyloid deposits in the brain parenchyma at about 11 months of age and β-amyloid deposits in larger cerebral vessels at about 18 months of age. The animals were deeply anaesthetized and transcardially perfused with 0.1 M phosphate-buffered saline (PBS) to flush the blood. Then, the brain was removed from the cranium and divided longitudinally. One hemisphere of the brain was shock-frozen and the other fixated by immersion into 4% paraformaldehyde. The immersion-fixated hemisphere was cryoprotected by soaking in 30% sucrose in PBS and mounted on a freezing microtome. The entire forebrain was cut into 40 μm transverse sections which were collected in PBS and used for the subsequent staining procedure. The neocortex samples from Alzheimer's disease patients were obtained from Brain-Net, Munich, Germany as frozen tissue, immersion-fixated in 4% paraformaldehyde during thawing, and subsequently treated like the mouse tissue.

Individual sections were stained with Congo Red using the following protocol:

Material:

- Amyloid dye Congo Red kit (Sigma-Aldrich; HT-60), consisting of alcoholic NaCl solution, NaOH solution and Congo Red solution
- staining cuvettes
- microscope slides SuperfrostPlus and coverslips
- Ethanol, Xylol, embedding medium

Reagents:

- NaOH diluted 1:100 with NaCl solution yields alkaline saline
- alkaline saline diluted 1:100 with Congo Red solution yields alkaline Congo Red solution (prepare no more than 15 min before use, filtrate)
- mount sections on slide and allow them to dry
- incubate slide in staining cuvette, first for 30-40 minutes in alkaline saline, then for 30-40 minutes in alkaline Congo Red solution
- rinse three times with fresh ethanol and embed over xylol

Staining was first photographed using a Zeiss Axioplan microscope (Zeiss, Jena, Germany) and evaluated qualitatively. Red color indicated amyloid deposits both in the form of plaques and in larger meningeal vessels. Later on, evaluation of antibody staining focused on these structures.

Staining was performed by incubating the sections with a solution containing 0.07 - 0.7 µg/ml of the respective antibody in accordance with the following protocol:

Materials:

- TBST washing solution (Tris Buffered Saline with Tween 20; 10x concentrate; DakoCytomation S3306, DAKO, Hamburg, Germany) 1:10 in Aqua bidest.)
- 0.3% H_2O_2 in methanol
- donkey serum (Serotec, Düsseldorf, Germany), 5% in TBST, as blocking serum
- monoclonal mouse-anti-globulomer antibodies diluted at given concentrations in TBST
- secondary antibody: biotinylated donkey-anti-mouse antibody (Jackson Immuno / Dianova, Hamburg, Germany; 715-065-150; diluted 1:500 in TBST)
- StreptABComplex (DakoCytomation K 0377, DAKO, Hamburg, Germany)
- Peroxidase Substrate Kit diaminobenzidine (=DAB; SK-4100; Vector Laboratories, Burlingame, CA, USA)
- SuperFrost Plus microscope slides and coverslips
- xylol free embedding medium (Medite, Burgdorf, Germany; X-tra Kitt)

Procedure:

- transfer floating sections into ice-cold 0.3% H_2O_2 and incubate for 30 min
- wash for 5 min in TBST buffer
- incubate with donkey serum/TBST for 20 minutes
- incubate with primary antibody for 24 hours at room temperature
- wash in TBST buffer for 5 minutes
- incubate with blocking serum for 20 minutes
- wash in TBST buffer for 5 minutes
- incubate with secondary antibody for 60 minutes at ambient temperature
- wash in TBST buffer for 5 minutes

- incubate with StreptABComplex for 60 minutes at ambient temperature
- wash in TBST buffer for 5 minutes
- incubate with DAB for 20 minutes
- mount the section on slides, air-dry slides, dehydrate slides with alcohol and embed slides

Besides visual inspection of sections under the microscope, amyloid staining was additionally quantified by optically excising 10 randomly selected plaques from the histological images using the ImagePro 5.0 image analysis system and determining their average greyscale value. Optical density values (were calculated from the greyscale values by subtracting the mean background density of the stained material from the density of amyloid plaques (0% - no plaque staining above surrounding background, 100% - no transmission / maximal staining). The differences between between antibodies 6E10 / 4G8 and 6G1, 8C5 and 8F5, respectively, were tested for statistical significance with ANOVA.

Results:

All antibody-stained material described proved to be congophilic amyloid deposits (Fig. 7(A)). The globulomer-preferring antibodies 8F5 and 8C5 stained parenchymal and meningeal congophilic deposits of Aβ peptide significantly less than the antibodies 6G1 and 6E10 (Fig. 7(B)-(C),(H)). Quantitative analysis of parenchymal amyloid plaque staining revealed binding of all antibodies to plaques (statistically significant density above control), but binding of antibody 8F5 and 8C5 was significantly lower than binding of the reference antibody 6E10 (raised to N-terminal sequence of Aβ)

and equal or lower than reference antibody 4G8 (raised to N-terminal sequence of Aβ) (Fig. 7(D)-Fig. (G)).

Antibodies 8F5 and 8C5 bind less to amyloid deposits than antibodies which recognize Aβ monomer or part of the Aβ sequence. Treatment with antibodies binding to fibrillar Aβ peptide can lead to fast dissolution of amyloid plaques in brain tissue and a subsequent increase of soluble Aβ concentration, which in turn is thought to be neurotoxic and could lead to microhemorrhages, and/or a fast dissolution of vascular amyloid, which also could lead to microhemorrhages. Therefore, an antibody therapy that affects the soluble globulomer rather than the monomer is preferred.

WHAT IS CLAIMED IS:

1. An isolated antibody that binds with greater specificity to an amyloid beta (Aβ) protein globulomer than to an amyloid beta protein monomer.
2. The isolated antibody of claim 1 wherein said antibody is monoclonal.
3. The isolated antibody of claim 2 wherein the ratio of binding specificity to said globulomer and said monomer is at least 1.4.
4. The isolated antibody of claim 3 wherein said ratio is 1.4 to 16.9.
5. The isolated antibody of claim 4 wherein said amyloid beta protein monomer is selected from the group consisting of Aβ(1-42) monomer and Aβ(1-40) monomer.
6. The isolated antibody of claim 5 wherein said monoclonal antibody is produced by a hybridoma having American Type Culture Collection designation number PTA-7238 or PTA-7407.
7. A hybridoma having American Type Culture Collection designation number PTA-7238.
8. A monoclonal antibody (8F5) produced by said hybridoma of claim 7.
9. A monoclonal antibody comprising a variable heavy chain encoded by SEQ ID NO:1.
10. The monoclonal antibody of claim 9 wherein said antibody is human or humanized.
11. A monoclonal antibody comprising a variable light chain encoded by SEQ ID NO:2.
12. The monoclonal antibody of claim 11 wherein said antibody is human or humanized.
13. The monoclonal antibody of claim 11 further comprising a variable light heavy chain encoded by SEQ ID NO:1.

14. The monoclonal antibody of claim 13 wherein said antibody is human or humanized.

15. A monoclonal antibody comprising SEQ ID NO:3.

16. The monoclonal antibody of claim 15 wherein said antibody is human or humanized.

17. A monoclonal antibody comprising SEQ ID NO:4.

18. The monoclonal antibody of claim 17 wherein said antibody is human or humanized.

19. The monoclonal antibody of claim 17 further comprising SEQ ID NO:3.

20. The monoclonal antibody of claim 19 wherein said antibody is human or humanized.

21. An isolated antibody that binds with greater specificity to an amyloid beta protein globulomer than to an amyloid beta protein fibril.

22. The isolated antibody of claim 22 wherein said antibody is monoclonal.

23. The isolated antibody of claim 23 wherein said monoclonal antibody is produced by a hybridoma having American Type Culture Collection designation number PTA-7238 or PTA-7407.

24. A hybridoma having American Type Culture Collection designation number PTA-7407.

25. A monoclonal antibody (8C5) produced by said hybridoma of claim 24.

26. A monoclonal antibody comprising a variable heavy chain encoded by SEQ ID NO:11.

27. The monoclonal antibody of claim 26 wherein said antibody is human or humanized.

28. A monoclonal antibody comprising a variable light chain encoded by SEQ ID NO:12.

29. The monoclonal antibody of claim 28 wherein said antibody is human or humanized.

30. The monoclonal antibody of claim 28 further comprising a variable light chain encoded by SEQ ID NO:11.

31. The monoclonal antibody of claim 30 wherein said antibody is human or humanized.

32. A monoclonal antibody comprising SEQ ID NO:19.

33. The monoclonal antibody of claim 32 wherein said antibody is human or humanized.

34. A monoclonal antibody comprising SEQ ID NO:20.

35. The monoclonal antibody of claim 34 wherein said antibody is human or humanized.

36. A monoclonal antibody comprising a variable heavy chain, wherein said variable heavy chain comprises at least one complementarity determining region (CDR) selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

37. A monoclonal antibody comprising a variable light chain, wherein said variable light chain comprises at least one CDR selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18.

38. The monoclonal antibody of claim 37 further comprising a variable heavy chain, wherein said variable heavy chain comprises at least one CDR selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

39. A monoclonal antibody comprising a variable heavy chain, wherein said variable heavy chain comprises at least one complementarity determining region (CDR) selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

40. A monoclonal antibody comprising a variable light chain, wherein said variable light chain comprises at least one CDR selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

41. The monoclonal antibody of claim 40 further comprising a variable heavy chain, wherein said variable heavy chain comprises at least one CDR selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

42. A method of treating or preventing Alzheimer's Disease in a patient in need of said treatment or prevention comprising administering said isolated antibody of claim 1 or claim 6 to said patient in an amount sufficient to effect said treatment or prevention.

43. The method of claim 42 wherein said isolated antibody is administered *via* a route selected from the group consisting of intramuscular administration, intravenous administration and subcutaneous administration.

44. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with said isolated antibody of claim 1 or claim 6 for a time and under conditions sufficient for formation of antigen/antibody complexes; and

c. detecting presence of said antigen/antibody complexes in said sample, presence of said complexes indicating a diagnosis of Alzheimer's Disease in said patient.

45. The method of claim 44 wherein said antigen is a globulomer.

46. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with an antigen for a time and under conditions sufficient for the formation of antibody/antigen complexes;

c. adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow said conjugate to bind to the bound antibody, wherein said conjugate comprises said isolated antibody of claim 1 or claim 6, attached to a signal generating compound capable of generating a detectable signal; and

d. detecting the presence of an antibody which may be present in said biological sample, by detecting a signal generated by said signal generating compound, said signal indicating a diagnosis of Alzheimer's Disease in said patient.

47. The method of claim 46 wherein said antigen is a globulomer.

48. A method of diagnosing Alzheimer's Disease in a patient suspected of having Alzheimer's Disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with anti-antibody, wherein said anti-antibody is specific for said antibody of claim 1 or claim 6, for a time and under conditions sufficient to allow for formation of anti-antibody/antibody complexes, said complexes containing antibody present in said biological sample;

c. adding a conjugate to resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow said conjugate to bind to bound antibody, wherein said conjugate comprises an antigen, which binds to a signal

generating compound capable of generating a detectable signal; and

d. detecting a signal generated by said signal generating compound, said signal indicating a diagnosis of Alzheimer's Disease in said patient.

49. A composition comprising said isolated antibody of claim 1 or claim 6.

50. A method of preventing or treating Alzheimer's Disease in a patient in need of said prevention or treatment comprising the step of administering said composition of claim 49 to said patient in an amount sufficient to effect said prevention or treatment.

51. A vaccine comprising said isolated antibody of claim 1 or claim 6 and a pharmaceutically acceptable adjuvant.

52. A method of preventing or treating Alzheimer's Disease in a patient in need of said prevention or treatment comprising the step of administering said vaccine of claim 51 to said patient in an amount sufficient to effect said prevention or treatment.

53. A method of identifying compounds suitable for active immunization of a patient predicted to develop Alzheimer's Disease comprising the steps of:

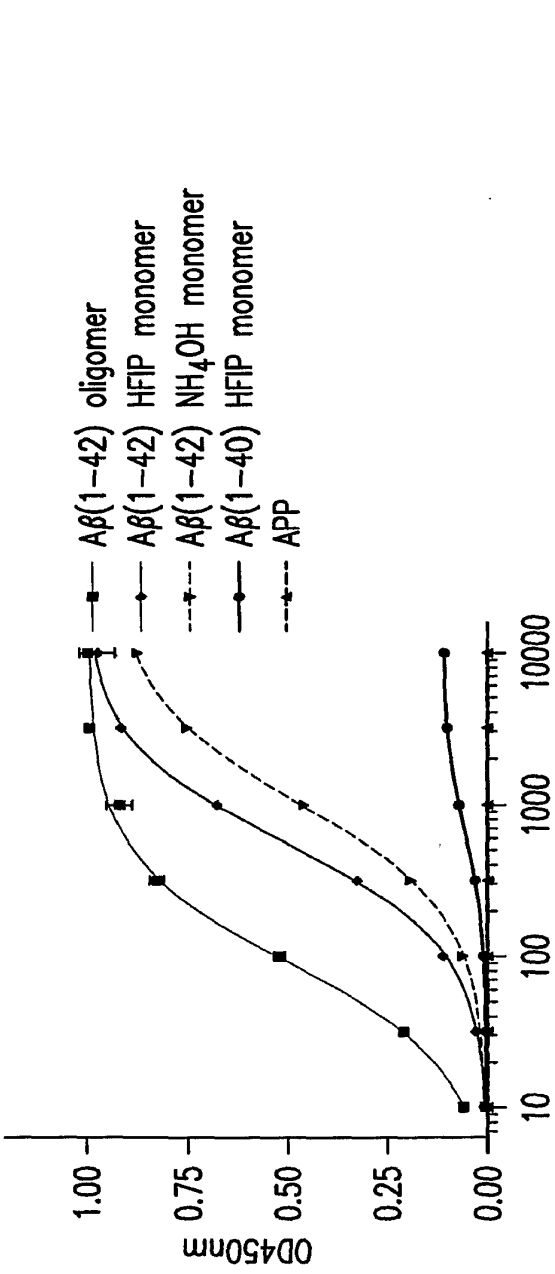
a) exposing one or more compounds of interest to said isolated antibody of claim 1 or claim 6 for a time and under conditions sufficient for said one or more compounds to bind to said isolated antibody of claim 1 or claim 6; and

b) identifying those compounds which bind to said isolated antibody of claim 1 or claim 6, said identified compounds to be used in active immunization in a patient predicated to develop Alzheimer's Disease.

54. A kit comprising: a) said isolated antibody of claim 1 or claim 6 and b) a conjugate comprising an antibody attached to a signal- generating compound, wherein said antibody of said conjugate is different from said isolated antibody.

55. A kit comprising: a) an anti-antibody to said isolated antibody of claim 1 or claim 6 and b) a conjugate comprising an antigen attached to a signal-generating compound.

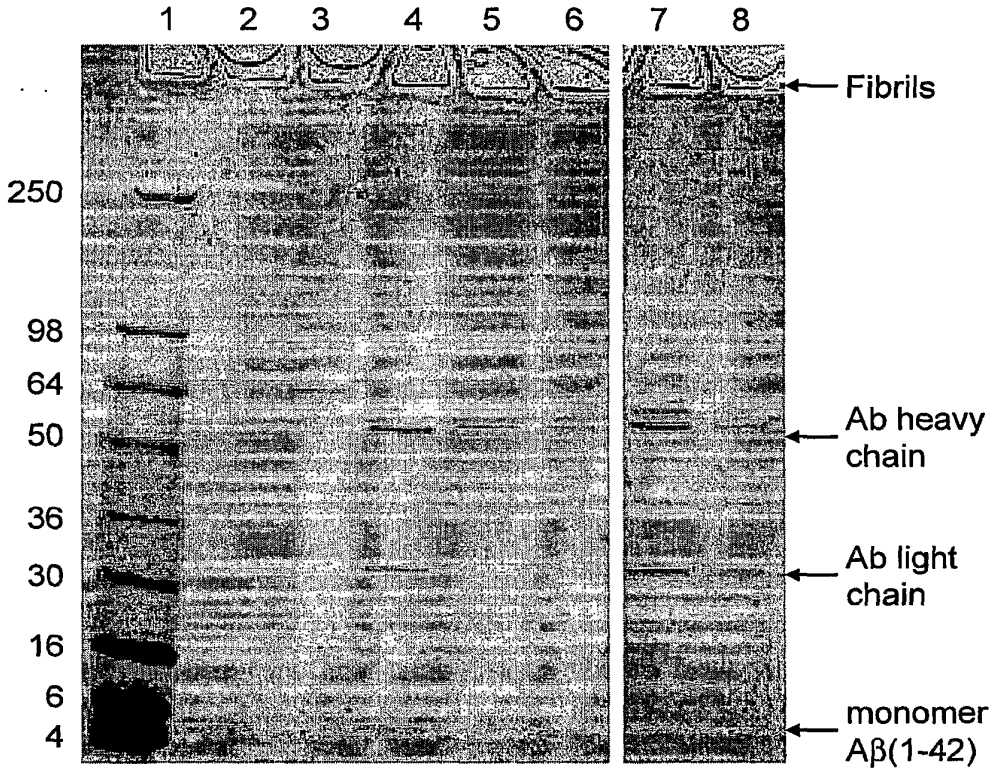
56. The kit of claim 55 wherein said antigen is a globulomer.



	Aβ(1-42) oligomer	Aβ(1-42) HFIP monomer	Aβ(1-42) NH_4OH monomer	Aβ(1-40) HFIP monomer
BOTTOM	-0.001218	0.004304	0.006727	0.004288
TOP	0.9926	1.002	0.9292	0.1116
LOGEC50	1.958	2.745	3.003	2.825
HILLSLOPE	1.215	1.270	1.204	1.375
EC50	90.74	555.8	1007	667.8

FIG. 1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5524



1. Marker
2. Fibril preparation control
3. Fibril preparation ; + mMAb 6E10 ; 20h 37°C ; supernatant
4. Fibril preparation ; + mMAb 6E10 ; 20h 37°C ; pellet
5. Fibril preparation ; + mMAb 4G8 ; 20h 37°C ; supernatant
6. Fibril preparation ; + mMAb 4G8 ; 20h 37°C ; pellet
7. Fibril preparation ; + mMAb 8F5 ; 20h 37°C ; supernatant
8. Fibril preparation ; + mMAb 8F5 ; 20h 37°C ; pellet

FIG.2

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EX-1045
5525

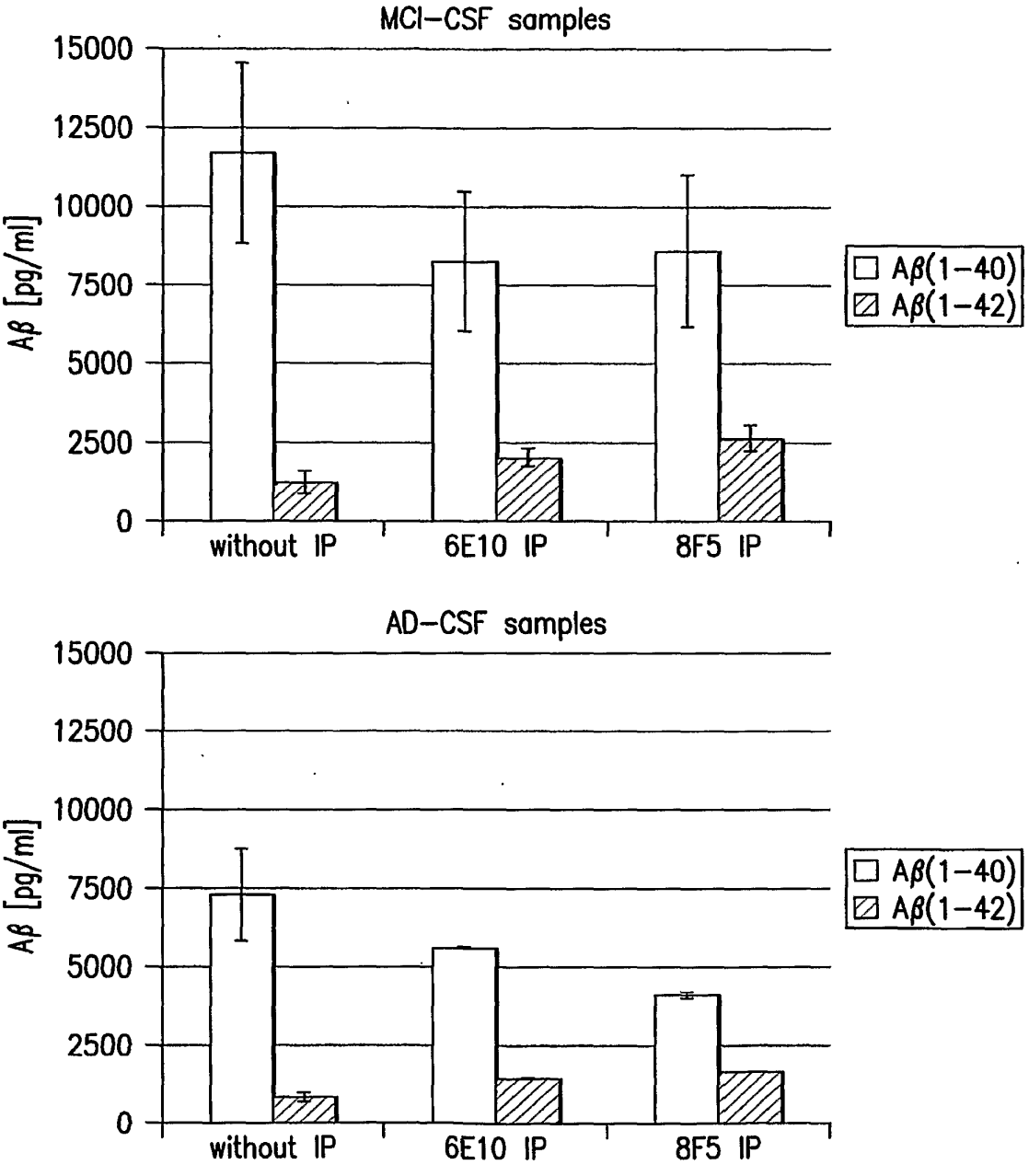
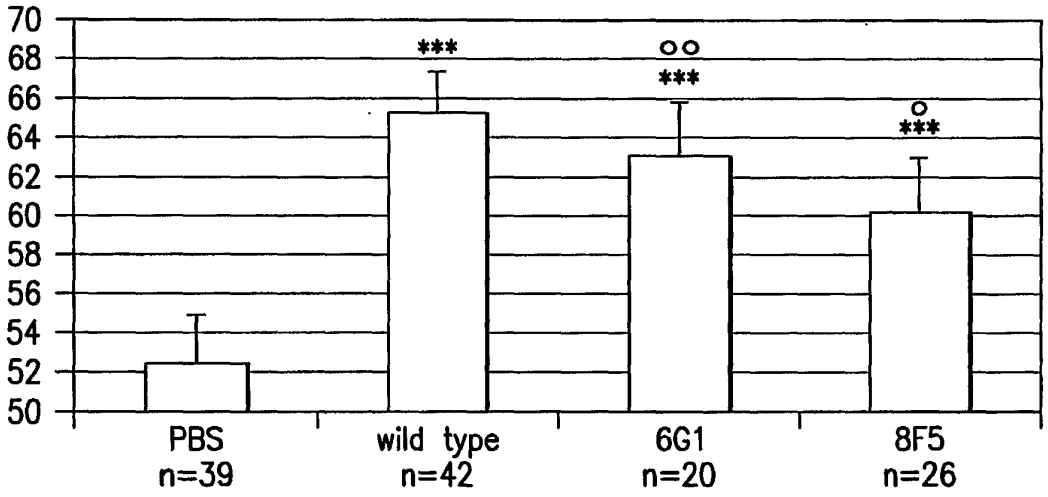


FIG.3

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5526



*** $P<0.001$ vs. 50%
o $P<0.05$; oo $P<0.01$
post hoc t-test vs. PBS after $P<0.001$ in ANOVA

FIG.4

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5527

VH 8F5

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGCCC
TGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGGCATGTCT
TGGGTTCGCCAGACTCCAGACAAGAGGCTGGAATTGGTCGCAAGCATCAATA
GTAATGGTGGTAGCACCTATTATCCAGACAGTGTGAAGGGCCGATTCACCAT
CTCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGAGCAGTCTGAAG
TCTGAGGACACAGCCATGTATTACTGTGCAAGTGGTGACTACTGGGGCCAAG
GCTCCACTCTCACAGTCTCCTCA (SEQ ID NO:1)

FIG.5A

VL 8F5

GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCA
AGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTATATAGTAATGGAGACA
CCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC
TACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTG
GATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCT
GGGAGTTTATTTCTGCTCTCAAAGTACACATGTTCCTTGGACGTTCGGTGGAG
GCACCAAGCTAGAAATCAAACGG (SEQ ID NO:2)

FIG.5B

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EX-1045
5528

VH 8F5

EVQLVESGGGLVQPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLELVASINSN
GGSTYYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCASGDYWGQGST
LTVSS (SEQ ID NO:3)

CDR1 (VH) = SYGMS (SEQ ID NO:5)
CDR2 (VH) = SINSNGGSTYYPDSVKG (SEQ ID NO:6)
CDR3 (VH) = SGDY (SEQ ID NO:7)

FIG.6A

VL 8F5

DVVMTQTPLSLPVSLGDQASISCRSSQSLVYSNGDTYLHWYLQKPGQSPKLLIYK
VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLE
IKR (SEQ ID NO:4)

CDR1(VL) = RSSQSLVYSNGDTYLH (SEQ ID NO:8)
CDR2(VL) = KVSNRFS (SEQ ID NO:9)
CDR3(VL) = SQSTHVPWT (SEQ ID NO:10)

FIG.6B

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EX-1045
5529

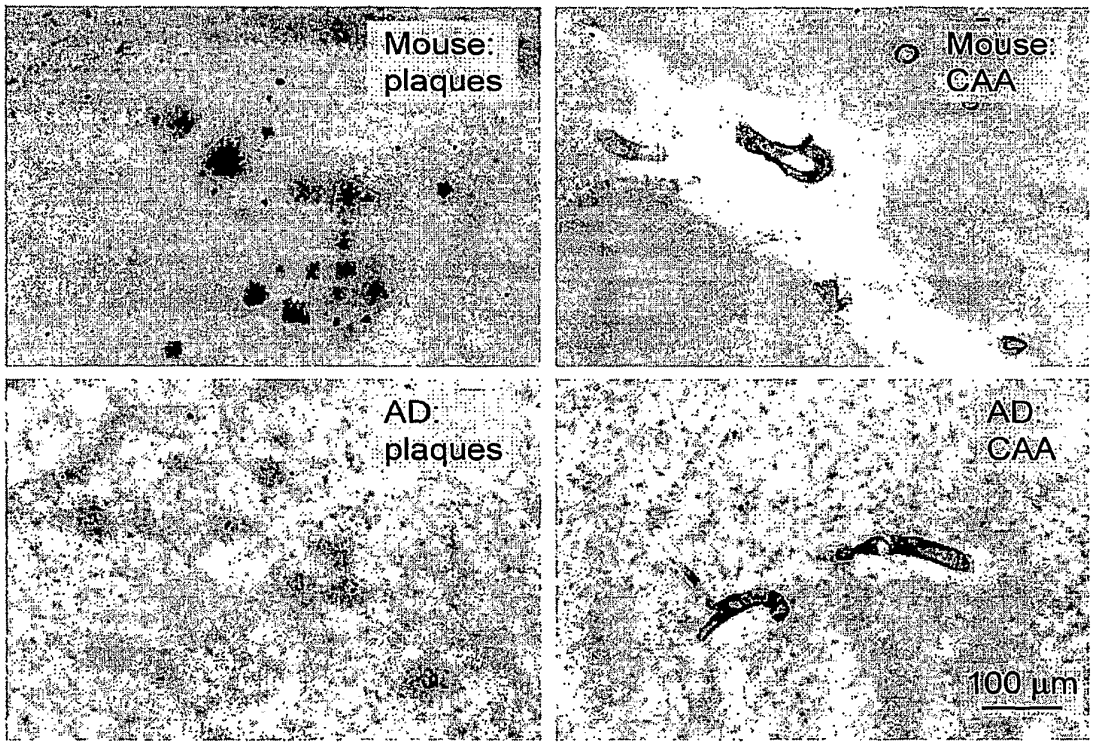


FIG.7A

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EX-1045
5530

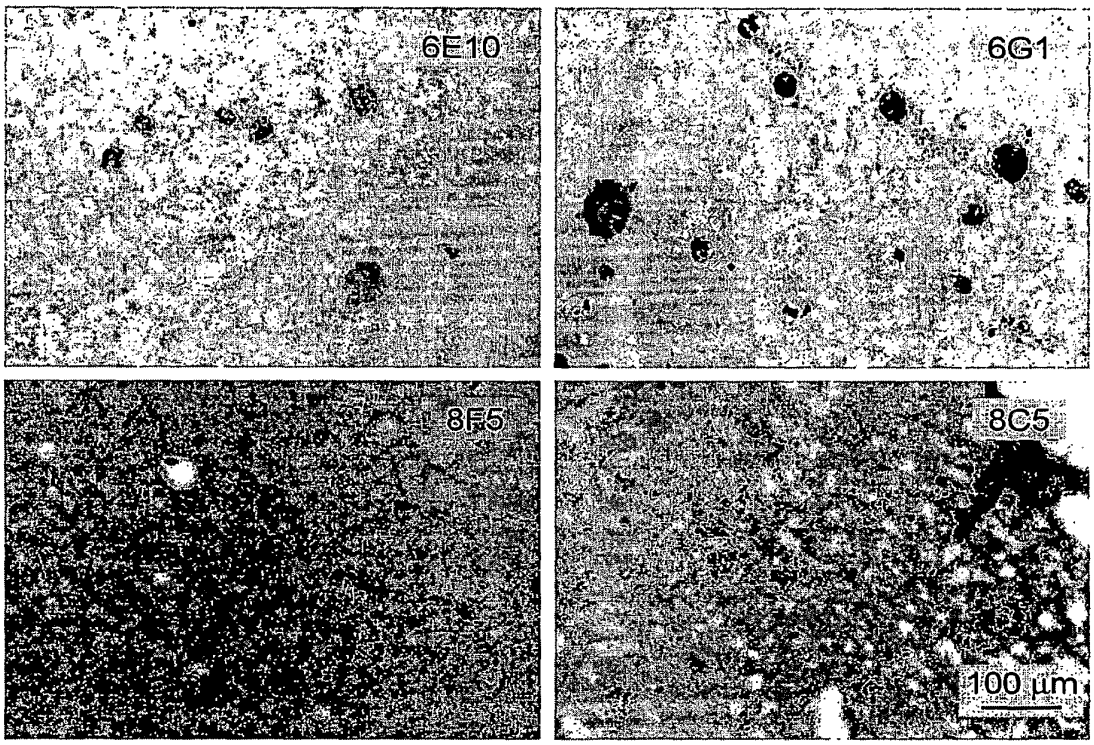


FIG.7B

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EX-1045
5531

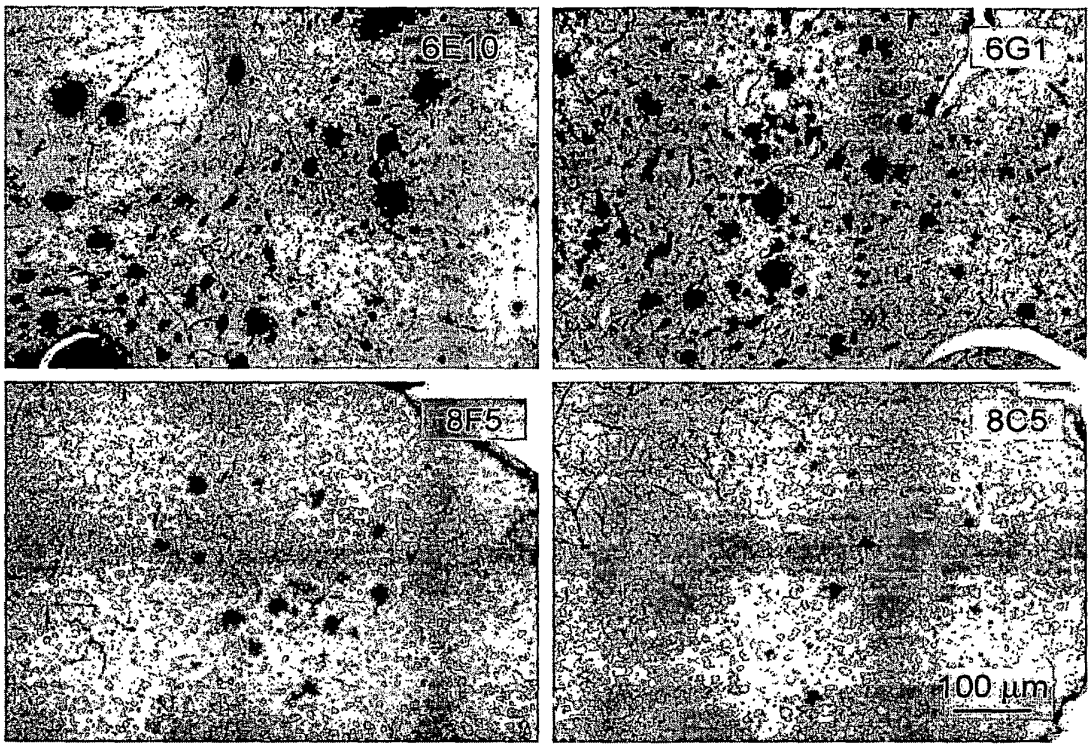


FIG.7C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5532

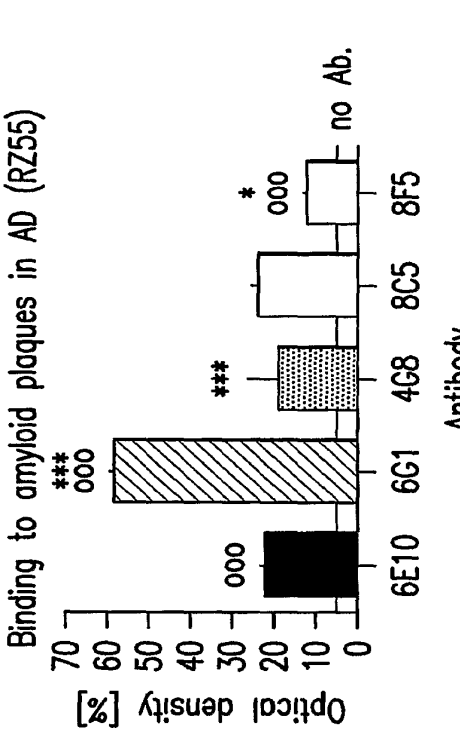


FIG.7F

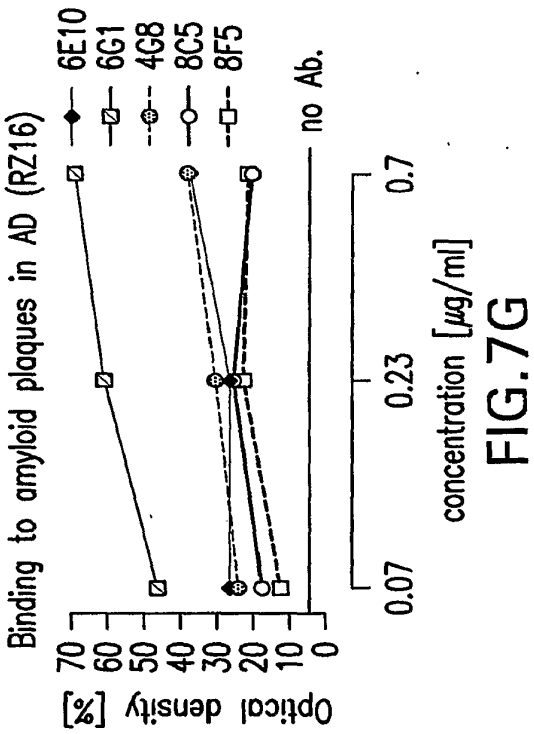


FIG.7G

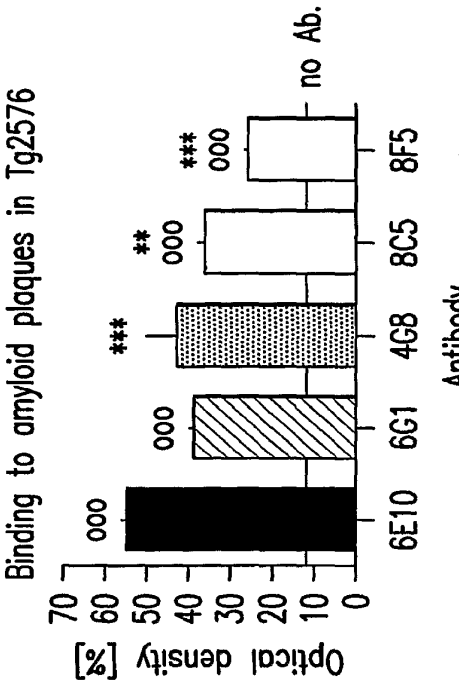


FIG.7D

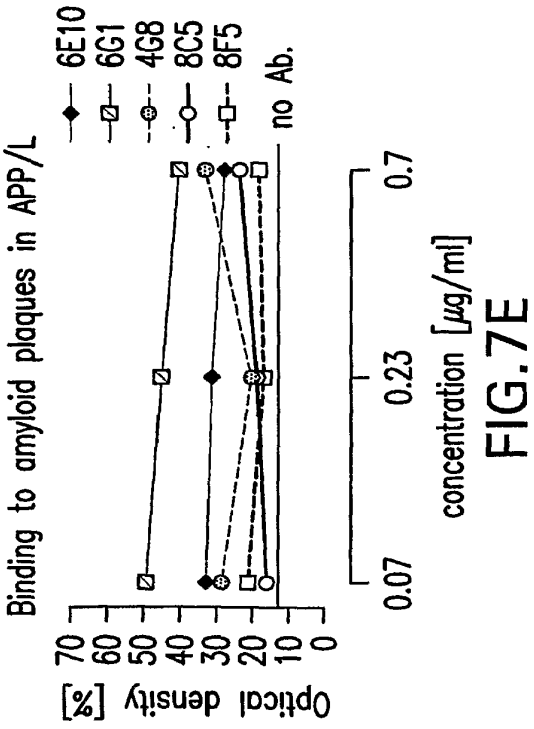


FIG.7E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5533

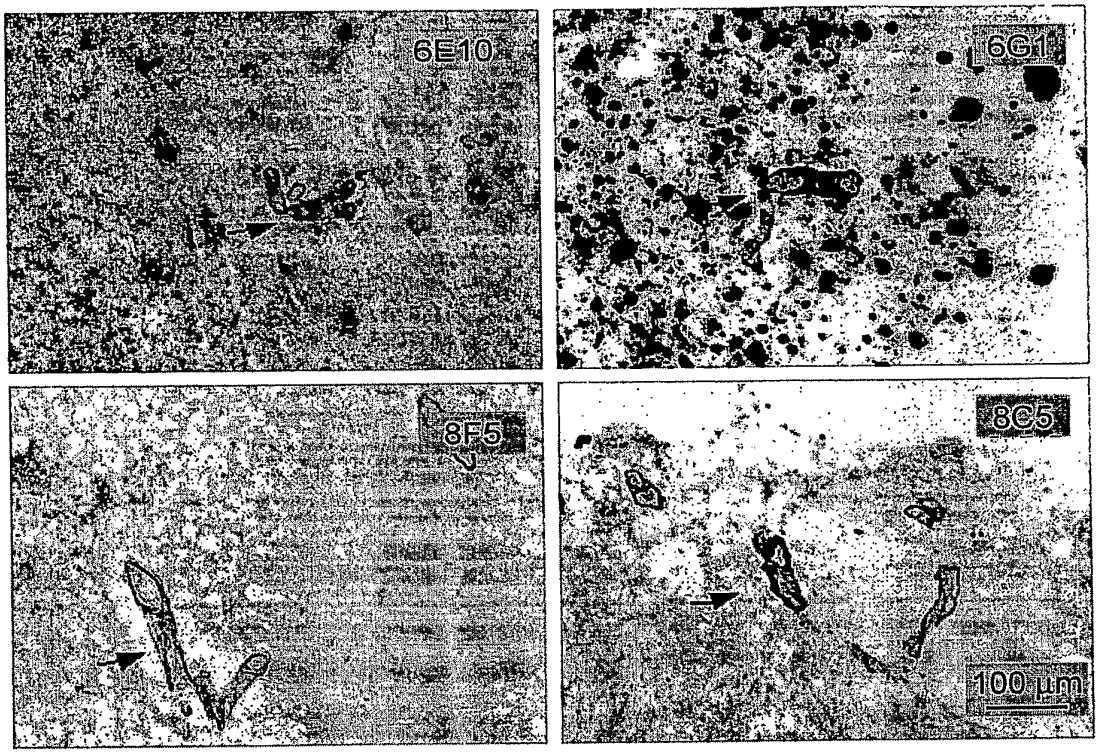
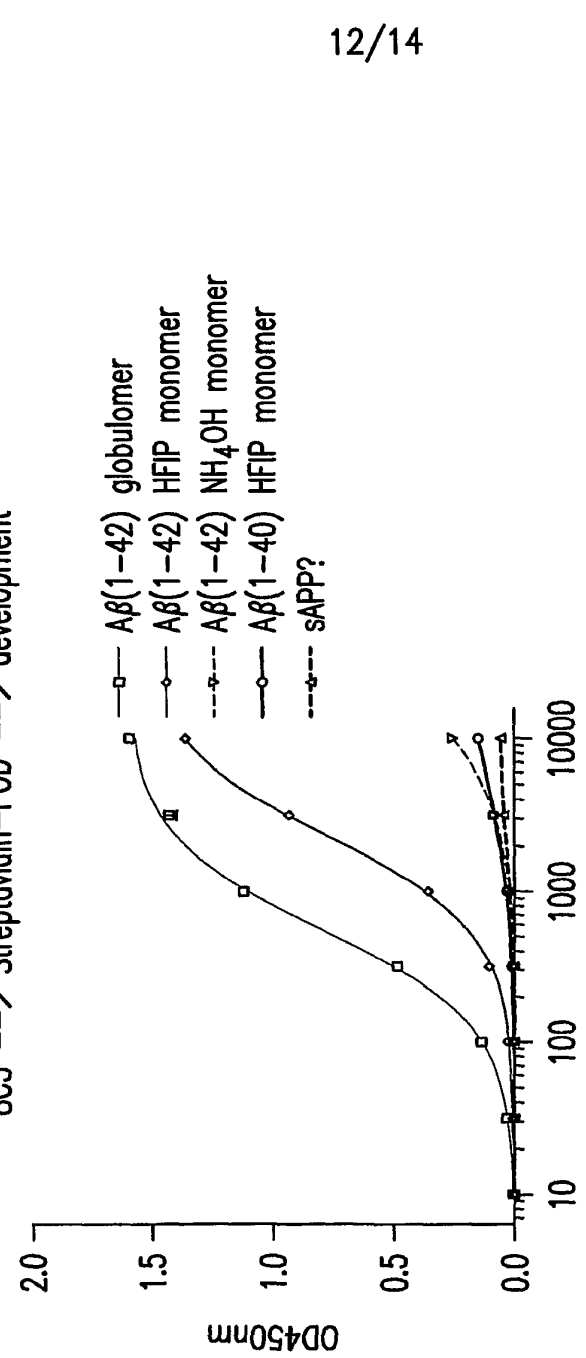


FIG.7H

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5534



	Aβ(1-42) globulomer	Aβ(1-42) HFIP monomer	Aβ(1-42) NH_4OH monomer	Aβ(1-40) HFIP monomer	sAPP?
BOTTOM	0.0003317	0.005757	-0.008508	0.0002754	0.0003000
TOP	1.601	1.545	1.137	0.2455	0.06415
LOGEC50	2.755	3.370	4.505	3.794	3.237
HILLSLOPE	1.369	1.377	1.052	0.9842	1.197
EC50	568.2	2346	31991	6222	1725

FIG.8

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5535

VH 8C5

GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCC
TGAAACTCTCCTGTACAGCCTCTGGATTCACTTTCAGTAGCTATGGCATGTCT
TGGGTTCGCCAGACTCCAGACAAGAGGCTGGAGTTGGTCGCAAGTATTAAAA
ATAATGGTGGTAGCACCTATTATCCAGACAGTTTGAAGGGCCGATTCACCAT
CTCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGAGCAGTCTGAAG
TCTGAGGACACAGCCATGTATTATTGTGCAAGTGGGGATTACTGGGGCCAAG
GCACCACTCTCACAGTCTCCTCA (SEQ ID NO:11)

FIG.9A

VL 8C5

GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCA
AGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACAGTAATGGAGAC
ACCTTTTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGAT
CTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTG
GATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCT
GGGAATTTATTTCTGCTCTCAGAGTATACATGTTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAATCAAACGG (SEQ ID NO:12)

FIG.9B

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5536

VH 8C5

EVQLVESGGGLVQPGGSLKLSCTASGFTFSSYGMSWVRQTPDKRLELVASIKNN
GGSTYYPDLSLKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCASGDYWGQGTT
LTVSS (SEQ ID NO:19)

CDR1 (VH) = SYGMS (SEQ ID NO:13)

CDR2 (VH) = SIKNNGGSTYYPDSLKG (SEQ ID NO:14)

CDR3 (VH) = SGDY (SEQ ID NO:15)

FIG.10A

VL 8C5

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGDTFLHWYLQKPGQSPKLLIYK
VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQSIHVPWTFGGGTKLEI
KR (SEQ ID NO:20)

CDR1 (VL) = RSSQSLVHSNGDTFLH (SEQ ID NO:16)

CDR2 (VL) = KVSNRFS (SEQ ID NO:17)

CDR3 (VL) = SQSIHVPWT (SEQ ID NO:18)

FIG.10B

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5537

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 June 2007 (21.06.2007)

PCT

(10) International Publication Number
WO 2007/068429 A1

(51) International Patent Classification:
C07K 16/18 (2006.01) *A61P 25/28* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/EP2006/011914

(22) International Filing Date:
11 December 2006 (11.12.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
05027090.9 12 December 2005 (12.12.2005) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** ANTIBODIES AGAINST AMYLOID BETA 4 WITH GLYCOSYLATED IN THE VARIABLE REGION

(57) **Abstract:** The present invention relates to a purified antibody molecule preparation being characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H). More specifically, a pharmaceutical and a diagnostic composition comprising said antibody molecule and antibody mixtures are provided which is/are capable of specifically recognizing the β-A4 peptide/Aβ4. The present invention relates in particular to a mixture of antibodies comprising one or two glycosylated antigen binding sites with a glycosylated asparagine (Asn) in the variable region of the heavy chain, i.e. mixtures of isoforms of antibodies which comprise a glycosylated Asn in the variable region of the heavy chain (V_H). Also disclosed are compositions or antibody preparations comprising the specifically glycosylated antibody isoforms. Furthermore, the pharmaceutical and diagnostic uses for these antibodies are provided. The antibody isoforms may for example be used in the pharmaceutical intervention of amyloidogenesis or amyloid-plaque formation and/or in the diagnosis of the same.

WO 2007/068429 A1

ANTIBODIES AGAINST AMYLOID BETA 4 WITH GLYCOSYLATED IN THE VARIABLE REGION

The present invention relates to a purified antibody molecule preparation being characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H). More specifically, a purified antibody molecule is provided which is capable of specifically recognizing the β-A4 peptide/Aβ4 and comprising a glycosylation in the variable region of the heavy chain (V_H). The present invention relates to a mixture of antibodies comprising one or two glycosylated antigen binding sites with a glycosylated asparagine (Asn) in the variable region of the heavy chain, i.e. mixtures of isoforms of antibodies which comprise a glycosylated Asn in the variable region of the heavy chain (V_H). Also disclosed are compositions or antibody preparations comprising the specifically glycosylated antibody isoforms. Furthermore, the pharmaceutical and diagnostic uses for these antibodies are provided. The antibody isoforms may for example be used in the pharmaceutical intervention of amyloidogenesis or amyloid-plaque formation and/or in the diagnosis of the same.

About 70% of all cases of dementia are due to Alzheimer's disease which is associated with selective damage of brain regions and neural circuits critical for cognition. Alzheimer's disease is characterized by neurofibrillary tangles in particular in pyramidal neurons of the hippocampus and numerous amyloid plaques containing mostly a dense core of amyloid deposits and defused halos.

The extracellular neuritic plaques contain large amounts of a pre-dominantly fibrillar peptide termed "amyloid β", "A-beta", "Aβ4", "β-A4" or "Aβ"; see Selkoe (1994), Ann. Rev. Cell Biol. 10, 373-403, Koo (1999), PNAS Vol. 96, pp. 9989-9990, US 4,666,829 or Glenner (1984), BBRC 12, 1131. This amyloid β is derived from "Alzheimer precursor protein/β-amyloid precursor protein" (APP). APPs are integral membrane glycoproteins (see Sisodia (1992), PNAS Vol. 89, pp. 6075) and are endoproteolytically cleaved within the Aβ sequence by a plasma membrane protease, α-secretase (see Sisodia (1992), loc. cit.). Furthermore, further secretase activity, in particular β-secretase and γ-secretase activity leads to the extracellular release of amyloid-β (Aβ) comprising either 39 amino acids (Aβ39), 40 amino acids (Aβ40), 42 amino

acids (Aβ42) or 43 amino acids (Aβ43); see Sinha (1999), PNAS 96, 11094-1053; Price (1998), Science 282, 1078 to 1083; WO 00/72880 or Hardy (1997), TINS 20, 154.

It is of note that Aβ has several naturally occurring forms, whereby the human forms are referred to as the above mentioned Aβ39, Aβ40, Aβ41, Aβ42 and Aβ43. The most prominent form, Aβ42, has the amino acid sequence (starting from the N-terminus): DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 3). In Aβ41, Aβ40, Aβ39, the C-terminal amino acids A, IA and VIA are missing, respectively. In the Aβ43-form an additional threonine residue is comprised at the C-terminus of the above depicted sequence (SEQ ID NO: 3).

The time required to nucleate Aβ40 fibrils was shown to be significantly longer than that to nucleate Aβ42 fibrils; see Koo, loc. cit. and Harper (1997), Ann. Rev. Biochem. 66, 385-407. As reviewed in Wagner (1999), J. Clin. Invest. 104, 1239-1332, the Aβ42 is more frequently found associated with neuritic plaques and is considered to be more fibrillogenic in vitro. It was also suggested that Aβ42 serves as a "seed" in the nucleation-dependent polymerization of ordered non-crystalline Aβ peptides; Jarrett (1993), Cell 93, 1055-1058.

Modified APP processing and/or the generation of extracellular plaques containing proteinaceous depositions are not only known from Alzheimer's pathology but also from subjects suffering from other neurological and/or neurodegenerative disorders. These disorders comprise, inter alia, Down's syndrome, Hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, ALS (amyotrophic lateral sclerosis), Creutzfeld Jacob disease, HIV-related dementia and motor neuropathy.

Until now, only limited medical intervention schemes for amyloid-related diseases have been described. For example, cholinesterase inhibitors like galantamine, rivastigmine or donepezil have been discussed as being beneficial in Alzheimer's patients with only mild to moderate disease. However, also adverse events have been reported due to cholinergic action of these drugs. While these cholinergic-enhancing treatments do produce some symptomatic benefit, therapeutic response is not satisfactory for the majority of patients treated. It has been estimated that significant cognitive improvement occurs in only about 5% of treated patients and there is little evidence that treatment significantly alters the course of this progressive disease.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5540

Consequently, there remains a tremendous clinical need for more effective treatments and in particular those which may arrest or delay progression of the disease.
Also NMDA-receptor antagonists, like memantine, have been employed more recently. However, adverse events have been reported due to the pharmacological activity. Further, such a treatment with these NMDA-receptor antagonists can merely be considered as a symptomatic approach and not a disease-modifying one

Also immunomodulation approaches for the treatment of amyloid-related disorders have been proposed. WO 99/27944 discloses conjugates that comprise parts of the Aβ peptide and carrier molecules whereby said carrier molecule should enhance an immune response. Another active immunization approach is mentioned in WO 00/72880, wherein also Aβ fragments are employed to induce an immune response.

Also passive immunization approaches with general anti-Aβ antibodies have been proposed in WO 99/27944 or WO 01/62801 and specific humanized antibodies directed against portions of Aβ have been described in WO 02/46237, WO 02/088306 and WO 02/088307. WO 00/77178 describes antibodies binding a transition state adopted by β-amyloid during hydrolysis. WO 03/070760 discloses antibody molecules that recognize two discontinuous amino acid sequences on the Aβ peptide.

WO 03/016466 describes a humanized anti-Aβ antibody which is modified in order to avoid any potential glycosylation in its heavy chain, since a glycosylation in variable region(s) of antibodies has been postulated in Wallick (1988) J. Exp. Med. 168, 1099-1109.

The technical problem underlying the present invention is to provide efficacious means and methods in the medical management of amyloid disorders, in particular means and methods for the reduction of detrimental amyloid plaques in patients in need of a (corresponding) medical intervention.

In a first aspect, the present invention provides a purified antibody molecule being characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H). The inventive, purified antibody or the antibody composition as provided herein is in particular directed against Aβ and/or a fragment of Aβ. The purified antibody molecule as provided herein and in particular the antibody composition or antibody preparation of the invention is useful in the preparation of a pharmaceutical or diagnostic

composition for the treatment, amelioration and for prevention of a disease associated with amyloidosis and/or amyloid plaque formation. An example of such disease is Alzheimer's disease.

In context of this invention it was surprisingly found that purified antibody molecules, wherein at least one antigen binding site comprises an N-linked glycosylation in the variable region of the heavy chain, is particularly useful in e.g. the reduction of amyloid plaques. Furthermore, it has been found in context of this invention that the glycosylated antibodies or antibody compositions as provided herein are particularly useful and efficacious in crossing the blood-brain barrier/blood brain border in vivo as illustrated by very efficacious plaque binding.

This is in stark contrast to the teachings of the prior art. WO 03/016466 discloses antibodies that are specifically engineered to lack an N-glycosylation site in the heavy chain and it is taught that glycosylation in variable region framework has negative effect on antibody binding affinity. It is taught in the prior art that the described anti-Aβ antibody in a deglycosylated form of the heavy chain variable CDR2 region has a markedly higher affinity for synthetic and purified Aβ peptide in vitro.

Accordingly, the present invention relates to an improved, purified antibody molecule or an antibody preparation, in particular an antibody molecule preparation that is directed against the Aβ4/Aβ peptide (amyloid β) and is highly efficient in vivo. The improvement of the present antibody molecule/antibody preparation lies in the provision of purified antibody molecules which comprise in at least one of their variable regions in the heavy chain an N-glycosylation, e.g. in the CDR2 region of said variable region of the heavy chain. As mentioned above, this is in contrast to the prior art like WO 03/016466 that teaches that such an N-glycosylation has to be avoided in antibodies directed against, e.g. Aβ.

Examples of an antibody molecule of the present invention are immunoglobulin molecules, e.g. IgG molecules. IgGs are characterized in comprising two heavy and two light chains (illustrated for example in figure 14) and these molecules comprise two antigen binding sites. Said antigen binding sites comprise "variable regions" consisting of parts of the heavy chains (V_H) and parts of the light chains (V_L). The antigen-binding sites are formed by the juxtaposition of the V_H and V_L domains. For general information on antibody molecules or immunoglobulin molecules see also common textbooks, like Abbas "Cellular and Molecular Immunology", W.B. Sounders Company (2003).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5542

In one aspect, for example in provision of an immunoglobulin molecule as characterized in this invention, an antibody is described wherein **one** antigen binding site comprises a glycosylated asparagine (Asn) in the corresponding variable region of the heavy chain (V_H). Said antibody is hereinafter referred to as "mono-glycosylated ANTIBODY"; see also figure 14.

In another aspect, an immunoglobulin molecule is provided wherein **both** antigen binding sites comprise a glycosylated asparagine (Asn) in the variable region of corresponding heavy chains (V_H). Said antibody molecule is hereinafter referred to as "double-glycosylated ANTIBODY", see figure 14.

An immunoglobulin wherein **no** antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H) is hereinafter referred to as "non-glycosylated ANTIBODY".

The mono-glycosylated ANTIBODY, the double-glycosylated ANTIBODY and the non-glycosylated ANTIBODY may comprise the identical amino acid sequences or different amino acid sequences. The term "ANTIBODY" comprises, accordingly, antibody molecules, in particular recombinantly produced antibody molecules, like immunglobulins. However, as discussed below, the term "ANTIBODY molecule(s)" also comprises known isoforms and modifications of immunoglobulins, like single-chain antibodies or single chain Fv fragments (scAB/scFv) or bispecific antibody constructs, said isoforms and modifications being characterized as comprising at least one glycosylated V_H region as defined herein. A specific example of such an isoform or modification may be a sc (single chain) antibody in the format V_H-V_L or V_L-V_H, wherein said V_H comprises the herein described glycosylation. Also bispecific scFvs are envisaged, e.g. in the format V_H-V_L-V_H-V_L, V_L-V_H-V_H-V_L, V_H-V_L-V_L-V_H, which comprise the herein described glycosylation in the CDR-2 region.

In context of this invention, the term "ANTIBODY" in capital letters is employed in order to provide for better lucidity. However, the term "antibody" used in small letters is also used in context of this application. "ANTIBODY"/"ANTIBODIES"/"antibody" and "antibodies" are used interchangeably.

The mono-glycosylated ANTIBODY and the double-glycosylated ANTIBODY are herein before referred to as "glycosylated ANTIBODY isoforms". A purified antibody molecule characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5543

in the variable region of the heavy chain (V_H) is a mono-glycosylated ANTIBODY which is free of or to a very low extent associated with an isoform selected from a double-glycosylated ANTIBODY and a non-glycosylated ANTIBODY, i.e. a "purified mono-glycosylated ANTIBODY". A double-glycosylated ANTIBODY in context of this invention is free of or to a very low extent associated with an isoform selected from a mono-glycosylated ANTIBODY and a non-glycosylated ANTIBODY, i.e. a "purified double-glycosylated ANTIBODY".

The term "which is free of or to a very low extent" denotes the complete absence of the respective other (glycosylation) isoforms or a presence of another (glycosylated) isoform in a concentration of at the most 10 %, e.g. at the most 5%, e.g. at the most 4%, e.g. at the most 3%, e.g. at the most 2%, e.g. at the most 1%, e.g. at the most 0.5%, e.g. at the most 0.3%, e.g. at the most 0.2%. Further information in this regard is provided below and in the appended examples.

In context of this invention, the term "mono-glycosylated antibody(ies)" or "mono-glycosylated ANTIBODY(IES)" relates to antibody molecules comprising an N-glycosylation in one (V_H)-region of an individual antibody molecule, e.g. of an immunoglobulin, e.g. an IgG, e.g. of an IgG1. For example, said "mono-glycosylated form", comprises a glycosylation on one variable region of the heavy chain e.g. at position asparagine "Asn 52" as defined below. This "mono-glycosylated IgG1-form or mono-glycosylated isoform" may also comprise, as illustrated herein, the glycosylation in the well conserved glycosylation site in the Fc-part, for example asparagine Asn 306 in the non-variable Fc-part.

The term "double-glycosylated antibody(ies)" or "double-glycosylated ANTIBODY(IES)" in the meaning of this invention comprises the herein defined glycosylation on both variable regions of the heavy chain (V_H)-region. Again, this "double glycosylated form", comprises a glycosylation on the variable region of both heavy chains, in particular at position asparagine Asn 52 as detailed below and as exemplified in the appended examples. This "double-glycosylated IgG1-form or double-glycosylated isoform" may also comprise, as illustrated herein, the glycosylation in the well conserved glycosylation site in the non-variable/constant Fc-part, in particular on position 306 of the exemplified immunoglobulin. Appended figure 14 illustrates corresponding antibody molecules.

Antibodies devoid of such a post-translational modification in the variable region, e.g. in both variable regions of the heavy chain (both (V_H)-regions) are, in context of this invention considered as a "non-glycosylated form", comprising no glycosylation in the variable region of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5544

the heavy chain. Yet, this "non-glycosylated form" may nevertheless comprise (a) glycosylation(s) in the constant region (C-region) of the antibody, for example, and most commonly at the well conserved glycosylation site of the Fc-part, in particular the asparagine (Asn) 306 in the non-variable/constant Fc-part as defined herein, see also SEQ ID NO: 6.

The glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H) may be in the complementarity determining region 2 (CDR2 region). Said glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H) may be in position 52 of the variable region as defined below and as shown in SEQ ID No. 2 (or in position 52 of SEQ ID NO: 6 comprising also the Fc-part of an antibody heavy chain as disclosed herein).

ANTIBODY isoforms may also comprise (a) further glycosylation(s) in the constant/non-variable part of the antibody molecules, e.g. in the Fc-part of an IgG, e.g. in the Fc-part in an IgG1. Said glycosylation in the Fc-part relates to a well conserved glycosylation, being characterized in located on position Asn306 of the heavy chain, e.g., in accordance with the following sequence:

QVELVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINASGTRT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVRYFDV
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 6)

This sequence is also depicted herein below and the CDRs, CH-regions, heavy regions as well as two N-glycosylation sites (N52 and N306) are indicated:

QVELVESGGGLVQPGGSLRLSCAAS GFTFSSYAMS WVRQAPGKGLEWVS
AINASGTRTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
GKGNTHKPYGYVRYFDV WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKV*EPKSCDKTHTCP*PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS

HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 6)

framed:CDR1, 2, 3

underlined: CH1

italics: hinge

underlined twice: CH2

dotted underlined: CH3

bold N: N-linked glycosylation sites

The IgG-Fc region of the antibodies of this invention may be a homodimer comprised of inter-chain disulphide bonded hinge regions, glycosylated C_H2 domains, bearing N-linked oligosaccharide at asparagine 306 (Asn-306) of the C_H2 and non-covalently paired C_H3 domains. The oligosaccharide of the glycosylation at Asn-306 is of the complex biantennary type and may comprise a core heptasaccharide structure with variable addition of outer arm sugars.

The oligosaccharide influences or determines Fc structure and function (Jefferis (1998) Immunol Rev. 163, 50-76). Effector functions, numbering particular specific IgG-Fc/effector ligand interactions have been discussed (Jefferis (2002) Immunol Lett. 82(1-2), 57-65 and Krapp (2003) J Mol Biol. 325(5), 979-89). This conserved Fc-position Asn-306 corresponds to "Asn-297" in the Kabat-system (Kabat (1991) Sequences of Proteins of Immunological Interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda MD.)

The exemplified heavy chain may be encoded by the following sequence:

caggtggaattggtggaaagcggcggcggcctggtgcaaccgggcggcagcctgcgtctgagctgcgcggcctccggatttacctttag
cagctatgcgatgagctgggtgcgccaagcccctgggaagggtctcgagtgggtgagcgctattaatgcttctggtactcgtacttattatgc
tgattctgttaagggtcgttttaccatttcacgtgataattcgaaaaacaccctgtatctgcaaatgaacagcctgcgtgcggaagatacggcc
gtgtattattgcgcgcgtggtaagggtaatactcataagccttatggttatgttcgttattttgatgtttggggccaaggcaccctggtgacggtt
agctcagcctccaccaagggtccatcggtcttcccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacag
tcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc

ccagcaacaccaaggtggacaagaaagttgagcccagatatcgtgcgatatcgtgcaatcttgtgacaaaactcacacatgcccaccgtgc
ccagcacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagaca
aagccgcgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagt
acaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
gtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctaca
gcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaatga (SEQ ID NO: 5).

Said heavy chain may also comprise (in particular during its recombinant production) additional sequences, like "leader sequences". A corresponding example is encoded by the following sequence:

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtcctgtcc (followed by)
caggtggaattggtggaaagcggcggcggcctggtgcaaccgggcggcagcctgcgtctgagctgcgcggcctccggatttacctttag
cagctatgcgatgagctgggtgcgccaagcccctgggaagggtctcgagtgggtgagcgctattaatgcttctggtactcgtacttattatgc
tgattctgttaagggtcgttttaccatttcacgtgataattcgaaaaacaccctgtatctgcaaatgaacagcctgcgtgcggaagatacggcc
gtgtattattgcgcgcgtggtaagggtaatactcataagccttatggttatgttcgttattttgatgtttggggccaaggcaccctggtgacggtt
agctcagcctccaccaagggtccatcggtcttcccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacag
tcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc
ccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgg
ggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgt
gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatc
ccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagca
atgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggaca
agagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgt
ctccgggtaaatga (SEQ ID NO: 25)

the corresponding amino acid sequence would be:

MKHLWFFLLLVAAPRWVLS (followed by)
QVELVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINASGTRT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVRYFDV
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 26)

The above sequence also comprises a "signal peptide" said signal peptide is proteolytically cleaved by the host signalpeptidase during the secretory pathway during the production of the inventive antibody molecules in host cells, like CHO cells.

Alternatively, said heavy chain may be encoded by a nucleic acid sequence that is optimized for recombinant production as exemplified by the following sequence:

```
  1  atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtga
 51  ttcatggaga aatagagaga ctgagtgtga gtgaacatga gtgagaaaaa
101  ctggatttgt gtggcatttt ctgataacgg tgtccttctg tttgcaggtg
151  tccagtgt
```

followed by

```
            ca ggtggagctg gtggagtctg ggggaggcct ggtccagcct
201  ggggggtccc tgagactctc ctgtgcagcg tctggattca ccttcagtag
251  ctatgccatg agctgggtcc gccaggctcc aggcaagggg ctcgagtggg
301  tgtccgccat aaacgccagc ggtacccgca cctactatgc agactccgtg
351  aagggccgat tcaccatctc cagagacaat tccaagaaca cgctgtatct
401  gcaaatgaac agcctgagag ccgaggacac ggctgtgtat tactgtgcga
451  gaggcaaggg gaacacccac aagccctacg gctacgtacg ctactttgac
501  gtgtggggcc aaggaaccct ggtcaccgtc tcctcaggtg agtcctcaca
551  acctctctcc tgcggccgca gcttgaagtc tgaggcagaa tcttgtccag
601  ggtctatcgg actcttgtga gaattagggg ctgacagttg atggtgacaa
651  tttcagggtc agtgactgtc tggtttctct gaggtgagac tggaatatag
701  gtcaccttga agactaaaga ggggtccagg ggcttttctg cacaggcagg
```

751 gaacagaatg tggaacaatg acttgaatgg ttgattcttg tgtgacacca
801 agaattggca taatgtctga gttgcccaag ggtgatctta gctagactct
851 ggggtttttg tcgggtacag aggaaaaacc cactattgtg attactatgc
901 tatggactac tggggtcaag gaacctcagt caccgtctcc tcaggtaaga
951 atggcctctc caggtcttta tttttaacct ttgttatgga gttttctgag
1001 cattgcagac taatcttgga tatttgccct gagggagccg gctgagagaa
1051 gttgggaaat aaatctgtct agggatctca gagcctttag gacagattat
1101 ctccacatct ttgaaaaact aagaatctgt gtgatggtgt tggtggagtc
1151 cctggatgat gggataggga ctttggaggc tcatttgagg gagatgctaa
1201 aacaatccta tggctggagg gatagttggg gctgtagttg gagattttca
1251 gtttttagaa tgaagtatta gctgcaatac ttcaaggacc acctctgtga
1301 caaccatttt atacagtatc caggcatagg gacaaaaagt ggagtggggc
1351 actttcttta gatttgtgag gaatgttcca cactagattg tttaaaactt
1401 catttgttgg aaggagctgt cttagtgatt gagtcaaggg agaaaggcat
1451 ctagcctcgg tctcaaaagg gtagttgctg tctagagagg tctggtggag
1501 cctgcaaaag tccagctttc aaaggaacac agaagtatgt gtatggaata
1551 ttagaagatg ttgcttttac tcttaagttg gttcctagga aaaatagtta
1601 aatactgtga ctttaaaatg tgagagggtt ttcaagtact catttttta
1651 aatgtccaaa atttttgtca atcaatttga ggtcttgttt gtgtagaact
1701 gacattactt aaagtttaac cgaggaatgg gagtgaggct ctctcatacc
1751 ctattcagaa ctgactttta acaataataa attaagttta aaatattttt
1801 aaatgaattg agcaatgttg agttgagtca agatggccga tcagaaccgg
1851 aacacctgca gcagctggca ggaagcaggt catgtggcaa ggctatttgg
1901 ggaagggaaa ataaaaccac taggtaaact tgtagctgtg gtttgaagaa
1951 gtggttttga aacactctgt ccagccccac caaaccgaaa gtccaggctg
2001 agcaaaacac cacctgggta atttgcattt ctaaaataag ttgaggattc
2051 agccgaaact ggagaggtcc tcttttaact tattgagttc aacctttaa
2101 ttttagcttg agtagttcta gtttccccaa acttaagttt atcgacttct
2151 aaaatgtatt tagaattcga gctcggtaca gctttctggg gcaggccagg
2201 cctgaccttg gctttggggc agggaggggg ctaaggtgag gcaggtggcg
2251 ccagcaggtg cacacccaat gcccatgagc ccagacactg gacgctgaac
2301 ctcgcggaca gttaagaacc caggggcctc tgcgcctggg cccagctctg
2351 tcccacaccg cggtcacatg gcaccacctc tcttgcagcc tccaccaagg
2401 gcccatcggt cttccccctg gcaccctcct ccaagagcac ctctgggggc
2451 acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac
2501 ggtgtcgtgg aactcaggcg ccctgaccag cggcgtgcac accttcccgg
2551 ctgtcctaca gtcctcagga ctctactccc tcagcagcgt ggtgaccgtg

2601 ccctccagca gcttgggcac ccagacctac atctgcaacg tgaatcacaa
2651 gcccagcaac accaaggtgg acaagaaagt tggtgagagg ccagcacagg
2701 gagggagggt gtctgctgga agccaggctc agcgctcctg cctggacgca
2751 tcccggctat gcagccccag tccagggcag caaggcaggc cccgtctgcc
2801 tcttcacccg gagcctctgc ccgccccact catgctcagg gagagggtct
2851 tctggctttt tcccaggctc tgggcaggca caggctaggt gcccctaacc
2901 caggccctgc acacaaaggg gcaggtgctg ggctcagacc tgccaagagc
2951 catatccggg aggaccctgc ccctgaccta agcccacccc aaaggccaaa
3001 ctctccactc cctcagctcg gacaccttct ctcctcccag attccagtaa
3051 ctcccaatct tctctctgca gagcccaaat cttgtgacaa aactcacaca
3101 tgcccaccgt gcccaggtaa gccagcccag gcctcgccct ccagctcaag
3151 gcgggacagg tgccctagag tagcctgcat ccagggacag gccccagccg
3201 ggtgctgaca cgtccacctc catctcttcc tcagcacctg aactcctggg
3251 gggaccgtca gtcttcctct tccccccaaa acccaaggac accctcatga
3301 tctcccggac ccctgaggtc acatgcgtgg tggtggacgt gagccacgaa
3351 gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa
3401 tgccaagaca aagccgcggg aggagcagta caacagcacg taccgtgtgg
3451 tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac
3501 aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat
3551 ctccaaagcc aaaggtggga cccgtggggt gcgagggcca catggacaga
3601 ggccggctcg gcccaccctc tgccctgaga gtgaccgctg taccaacctc
3651 tgtccctaca gggcagcccc gagaaccaca ggtgtacacc ctgccccccat
3701 cccgggatga gctgaccaag aaccaggtca gcctgacctg cctggtcaaa
3751 ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc
3801 ggagaacaac tacaagacca cgcctcccgt gctggactcc gacggctcct
3851 tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg
3901 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac
3951 gcagaagagc ctctccctgt ccccgggcaa atga (SEQ ID NO: 23)

The "alternative" protein sequence as shown above as SEQ ID NO: 23 comprises the same coding sequence as the first alternative, however in a slightly different genomic organization, like additional introns and a slightly different "leader sequence"/"signal sequence". Said "leader sequence" may also comprise, as shown above an (additional) intron(s). The person skilled in the art is readily in a position to deduce in the sequence as shown herein the corresponding exon/intron structure by conventional methods.

The exemplified antibody described herein may also comprise a light chain, said light-chain may comprise or have the following amino acid sequence

DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVP
ARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 8)

which may be encoded by the following nucleic acid sequence:

gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcgagccagagcgtga
gcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatggcgcgagcagccgtgcaactggggtcc
cggcgcgttttagcggctctggatccggcacggattttaccctgaccattagcagcctggaacctgaagactttgcgacttattattgccttca
gatttataatatgcctattacctttggccagggtacgaaagttgaaattaaacgtacggtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg
ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggg
gagagtgttag (SEQ ID NO: 7).

Also the "light chain" of exemplified antibody described herein may comprise a "leader sequence" which is particularly useful in the technical production. A corresponding sequence may be (or may be comprised e.g. in a vector system) the following sequence:

atggtgttgcagacccaggtcttcatttctctgttgctctggatctctggtgcctacggg (followed by)
gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcgagccagagcgtga
gcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatggcgcgagcagccgtgcaactggggtcc
cggcgcgttttagcggctctggatccggcacggattttaccctgaccattagcagcctggaacctgaagactttgcgacttattattgccttca
gatttataatatgcctattacctttggccagggtacgaaagttgaaattaaacgtacggtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg
ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggg
gagagtgttag (SEQ ID NO: 27)

Said sequence encodes the following amino acid sequence

MVLQTQVFISLLLWISGAYG (followed by)
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVP
ARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRTVAAPSVFIFPPS

DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 28)

Alternatively, said light chain may also be encoded by a nucleic acid sequence that is optimized for recombinant production as exemplified by the following sequence:

```
   1  atggacatga gggtcctcgc tcagctcctg gggctcctgc tgctctgttt
  51  cccaggtaag gatggagaac actagcagtt tactcagccc agggtgctca
 101  gtactgcttt actattcagg gaaattctct tacaacatga ttaattgtgt
 151  ggacatttgt ttttatgttt ccaatctcag gcgccagatg t
```

followed by

```
                                                       gatatcgtg
 201  ttgacgcagt ctccagccac cctgtctttg tctccagggg aaagagccac
 251  cctctcctgc cgggccagtc agagtgttag cagcagctac ttagcctggt
 301  accagcagaa acctggccag gcgcccaggc tcctcatcta tggcgcatcc
 351  agcagggcca ctggcgtgcc agccaggttc agtggcagtg ggtctgggac
 401  agacttcact ctcaccatca gcagcctgga gcctgaagat ttcgcgacct
 451  attactgtct gcagatttac aacatgccta tcacgttcgg ccaagggacc
 501  aaggtggaaa tcaaacgtga gtagaattta aactttgcgg ccgcctagac
 551  gtttaagtgg gagatttgga ggggatgagg aatgaaggaa cttcaggata
 601  gaaaagggct gaagtcaagt tcagctccta aaatggatgt gggagcaaac
 651  tttgaagata aactgaatga cccagaggat gaaacagcgc agatcaaaga
 701  ggggcctgga gctctgagaa gagaaggaga ctcatccgtg ttgagtttcc
 751  acaagtactg tcttgagttt tgcaataaaa gtgggatagc agagttgagt
 801  gagccgtagg ctgagttctc tcttttgtct cctaagtttt tatgactaca
 851  aaaatcagta gtatgtcctg aaataatcat taagctgttt gaaagtatga
 901  ctgcttgcca tgtagatacc atgtcttgct gaatgatcag aagaggtgtg
 951  actcttattc taaaatttgt cacaaaatgt caaaatgaga gactctgtag
1001  gaacgagtcc ttgacagaca gctcaagggg ttttttttcct ttgtctcatt
1051  tctacatgaa agtaaatttg aaatgatctt ttttattata agagtagaaa
1101  tacagttggg tttgaactat atgttttaat ggccacggtt ttgtaagaca
1151  tttggtcctt tgttttccca gttattactc gattgtaatt ttatatcgcc
1201  agcaatggac tgaaacggtc cgcaacctct tctttacaac tgggtgacct
1251  cgcggctgtg ccagccattt ggcgttcacc ctgccgctaa gggccatgtg
1301  aacccccgcg gtagcatccc ttgctccgcg tggaccactt tcctgaggca
1351  cagtgatagg aacagagcca ctaatctgaa gagaacagag atgtgacaga
```

1401 ctacactaat gtgagaaaaa caaggaaagg gtgacttatt ggagatttca
1451 gaaataaaat gcatttatta ttatattccc ttattttaat tttctattag
1501 ggaattagaa agggcataaa ctgctttatc cagtgttata ttaaaagctt
1551 aatgtatata atcttttaga ggtaaaatct acagccagca aaagtcatgg
1601 taaatattct ttgactgaac tctcactaaa ctcctctaaa ttatatgtca
1651 tattaactgg ttaaattaat ataaatttgt gacatgacct taactggtta
1701 ggtaggatat ttttcttcat gcaaaaatat gactaataat aatttagcac
1751 aaaaatattt cccaatactt taattctgtg atagaaaaat gtttaactca
1801 gctactataa tcccataatt ttgaaaacta tttattagct tttgtgtttg
1851 acccttccct agccaaaggc aactatttaa ggacccttta aaactcttga
1901 aactacttta gagtcattaa gttatttaac cacttttaat tactttaaaa
1951 tgatgtcaat tccctttaa ctattaattt attttaaggg gggaaaggct
2001 gctcataatt ctattgtttt tcttggtaaa gaactctcag ttttcgtttt
2051 tactacctct gtcacccaag agttggcatc tcaacagagg ggactttccg
2101 agaggccatc tggcagttgc ttaagatcag aagtgaagtc tgccagttcc
2151 tcccaggcag gtggcccaga ttacagttga cctgttctgg tgtggctaaa
2201 aattgtccca tgtggttaca aaccattaga ccagggtctg atgaattgct
2251 cagaatattt ctggacaccc aaatacagac cctggcttaa ggccctgtcc
2301 atacagtagg tttagcttgg ctacaccaaa ggaagccata cagaggctaa
2351 tatcagagta ttcttggaag agacaggaga aaatgaaagc cagtttctgc
2401 tcttacctta tgtgcttgtg ttcagactcc caaacatcag gagtgtcaga
2451 taaactggtc tgaatctctg tctgaagcat ggaactgaaa agaatgtagt
2501 ttcagggaag aaaggcaata gaaggaagcc tgagaatacg gatcaattct
2551 aaactctgag ggggtcggat gacgtggcca ttctttgcct aaagcattga
2601 gtttactgca aggtcagaaa agcatgcaaa gccctcagaa tggctgcaaa
2651 gagctccaac aaaacaattt agaactttat taaggaatag ggggaagcta
2701 ggaagaaact caaaacatca agattttaaa tacgcttctt ggtctccttg
2751 ctataattat ctgggataag catgctgttt tctgtctgtc cctaacatgc
2801 cctgtgatta tccgcaaaca acacacccaa gggcagaact ttgttactta
2851 aacaccatcc tgtttgcttc tttcctcagg aactgtggct gcaccatctg
2901 tcttcatctt cccgccatct gatgagcagt tgaaatctgg aactgcctct
2951 gttgtgtgcc tgctgaataa cttctatccc agagaggcca aagtacagtg
3001 gaaggtggat aacgccctcc aatcgggtaa ctcccaggag agtgtcacag
3051 agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg
3101 agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcaccca
3151 tcagggcctg agctcgcccg tcacaaagag cttcaacagg ggagagtgtt
3201 **ag** (SEQ ID NO: 24)

The above "sequence" for an exemplified light chain has also a slightly different genomic structure. This "alternative sequence" comprises different and/or additional introns. Accordingly the embodiments describing for the "heavy chain" apply here mutatis mutandis.

In context of the present invention, the term "antibody molecule" relates to full immunoglobulin molecules, e.g. IgMs, IgDs, IgEs, IgAs or IgGs, like IgG1, IgG2, IgG2b, IgG3 or IgG4 as well as to parts of such immunoglobulin molecules, like Fab-fragments, Fab'-fragments, F(ab)2-fragements, chimeric F(ab)2 or chimeric Fab' fragments, chimeric Fab-fragments or isolated V_H- or CDR-regions (said isolated V_H- or CDR-regions being, e.g. to be integrated or engineered in corresponding "framework(s)") Also comprised in the term "antibody molecule" are diabodies and molecules that comprise an antibody Fc domain as a vehicle attached to at least one antigen binding moiety/peptide, e.g. peptibodies as described in WO 00/24782. Accordingly, and in context of this invention, the term "variable region of the heavy chain (V_H)" is not limited to a variable region in a full immunoglobulin but also relates to the corresponding parts of said variable region of the heavy chain (V_H), like the CDRs, either alone or in combination of the CDR1, 2, and/or 3 or the corresponding "framework" of the variable region. Therefore, an antibody molecule of the present invention may also be an antibody construct which comprises, as antigen binding site, the CDRs or at least one CDR of a given variable region of the glycosylated heavy chain (V_H). Said corresponding part of said variable region of the heavy chain (V_H) in the antibody construct of the invention is glycosylated as defined herein, e.g. comprises a glycosylated asparagine (Asn) in the antigen binding site. An example of such an "isolated part" a variable region of the heavy chain (V_H) is the herein exemplified CDR2 region comprised in SEQ ID NO: 12 (or encoded by a nucleic acid sequence as shown in SEQ ID NO: 11).
Furthermore, the term "antibody molecule" relates to modified and/or altered antibody molecules, like chimeric, humanized or fully humanized antibodies.

Said "fully humanized antibody" molecules are also characterized and described as "completely human" antibodies. All these antibodies can be generated by methods known in the art. For example, by phage display technology, recombinant antibody molecules may be generated due to the use of in vitro maturation which is the usage of a complete human immunoglobulin γ, subclass-1 framework (IgG1) as described by Knappik (2000) J Mol Biol. 296(1), 57-86. and Rauchenberger (2003) J Biol Chem. 278(40), 38194-205.

As documented in the appended examples, the term antibody, relates, e.g. to an IgG molecule and e.g. to an IgG1. The term also relates to modified or altered monoclonal or polyclonal antibodies as well as to recombinantly or synthetically generated/synthesized antibodies. The term also relates to intact antibodies as well as to antibody fragments/parts thereof, like, separated light and heavy chains, Fab, Fab/c, Fv, Fab', $F(ab')_2$. The term "antibody molecule" also comprises antibody derivatives, the bifunctional antibodies and antibody constructs, like single chain Fvs (scFv) or antibody-fusion proteins. Also envisaged are catalytic and/or proteolytic antibodies which comprise a glycosylated V_H domain, e.g. a glycosylated V_H-CDR as defined herein. The term "antibody molecule" relates also to recombinantly produced antibody molecules/antibody constructs which may comprise, besides one specificity (e.g. against Aβ/ Aβ), another or a further specificity. Such constructs may comprise, but a not limited to "bi-specific" or "tri-specific" constructs. Further details on the term "antibody molecule" of the invention are provided herein below.

As pointed out above, also envisaged are a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, an antibody-fusion protein, a cross-cloned antibody or a synthetic antibody comprising the herein defined glycosylation in at least one antigen binding site, e.g. in at least one variable region of a/the heavy chain defined herein and being glycosylated. When for example single-chain antibodies are produced, the herein defined "variable region of the heavy chain" is not limited to a heavy chain per se, but is meant to also relate to the corresponding parts derived from a heavy chain of a full antibody, e.g. a full immunoglobulin, like an IgG. Such parts may be the corresponding CDRs either alone or with parts of their corresponding framework. Furthermore, genetic variants of immunoglobulin genes are also envisaged in context of this invention. Genetic variants of, e.g., immunoglobulin heavy G chain subclass 1 (IgG1) may comprise the G1m(17) or G1m(3) allotypic markers in the CH1 domain, or the G1m(1) or the G1m(non-1) allotypic marker in the CH3 domain. Here, preferably an IgG1 of the Gm(17)(z) and Gm(1)(a) allotype is employed. The antibody molecule of the invention also comprises modified or mutant antibodies, like mutant IgG with enhanced or attenuated Fc-receptor binding or complement activation. In one embodiment, the antibody provided in accordance with this invention is a fully-humanized antibody or "completely human" antibody.

Accordingly, the antibodies of the invention may also comprise cross-cloned antibodies, i.e. antibodies comprising different antibody regions (e.g. CDR-regions) from one or more parental

or affinity-optimized antibody(ies) as described herein. These cross-cloned antibodies may be antibodies in several, different frameworks, e.g. an IgG-framework, e.g. a (human) IgG1-, IgG2a or an IgG2b-framework. For example, said antibody framework is a mammalian, e.g. a human framework. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, joined by three hypervariable domains known as complementarity determining regions (CDR1-3).

As used herein, a "human framework region" relates to a framework region that is substantially identical (about 85% or more, usually 90-95% or more) to the framework region of a naturally occurring human immunoglobulin. The framework region of an antibody (e.g. the combined framework regions of the constituent light and heavy chains) serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen. It is of note that not only cross-cloned antibodies described herein may be presented in a preferred (human) antibody framework, but also antibody molecules comprising CDRs from antibodies as described herein, may be introduced in an immunoglobulin framework. Examples for frameworks include IgG1, IgG2a and IgG2b. Most preferred are human frameworks and human IgG1 frameworks, such as the heavy chain of an ANTIBODY as shown in, inter alia, SEQ ID NO: 6.

In one embodiment ANTIBODY isoforms may comprise in the variable heavy chain region a **CDR1** comprising the following amino acids:

GFTFSSYAMS (SEQ ID NO: 10)

Said CDR1 may be encoded by the following nucleic acid sequence:

ggatttacctttagcagctatgcgatgagc (SEQ ID NO: 9)

ANTIBODY isoforms may comprise the following **CDR2** in the variable region of the heavy chain:

AINASGTRTYYADSVKG (SEQ ID NO: 12)

(**N:** N-linked glycosylation site at Asn-52 of a full heavy chain)

Said **CDR2** may be encoded by the following nucleic acid sequence:

gctattaatgcttctggtactcgtacttattatgctgattctgttaagggt (SEQ ID NO: 11)

The N-glycosylation in accordance with this invention is e.g. comprised in said CDR2 region and is located on the corresponding Asn52 of the variable region of the heavy chain, said variable region (V_H) being encoded by a nucleic acid molecule as shown in SEQ ID NO: 1 and having an amino acid sequence as shown in SEQ ID NO: 2.

Furthermore, ANTIBODY isoforms may comprise in their variable heavy chain region a **CDR3** comprising the following amino acid sequence:

GKGNTHKPYGYVRYFDV (SEQ ID NO: 14)

Said **CDR3** may be encoded by the following nucleic acid sequence:

ggtaagggtaatactcataagccttatggttatgttcgttattttgatgtt (SEQ ID NO: 13)

ANTIBODY isoforms may comprise a light (L) chain which may be characterized by the following CDRs:

CDR1: RASQSVSSSYLA (SEQ ID NO: 16)

agagcgagccagagcgtgagcagcagctatctggcg (SEQ ID NO: 15)

CDR2: GASSRAT (SEQ ID NO: 18)

ggcgcgagcagccgtgcaact (SEQ ID NO: 17)

CDR3: LQIYNMPI (SEQ ID NO: 20)

cttcagatttataatatgcctatt (SEQ ID NO: 19)

ANTIBODY isoforms may comprise additional potential glycosylation sites (as known in the art comprising the Asn-X-Ser/Thr motives) in the amino acid sequence of the heavy chains of the antibody, e.g. in the well conserved glycosylation site at Asn 306 in the non-variable Fc-part (corresponding to "Asn297" in the Kabat-system (Kabat (1991) Sequences of Proteins of Immunological Interest, 5th ed., Bethesda, Md.: National Center for Biotechnology Information, National Library of Medicine), said heavy chains being or comprising the sequence as provided above, namely in SEQ ID NO: 6 (as encoded by SEQ ID NO: 5).

In one embodiment of the present invention, the ANTIBODY isoforms are characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H), said V_H being encoded by

(a) a nucleic acid molecule comprising the nucleotide sequence as shown in SEQ ID NO: 1:

CAGGTGGAATTGGTGGAAAGCGGCGGCGGCCTGGTGCAACCGGGCGGC
AGCCTGCGTCTGAGCTGCGCGGCCTCCGGATTTACCTTTAGCAGCTATG
CGATGAGCTGGGTGCGCCAAGCCCCTGGGAAGGGTCTCGAGTGGGTGA
GCGCTATTAATGCTTCTGGTACTCGTACTTATTATGCTGATTCTGTTAAG
GGTCGTTTTACCATTTCACGTGATAATTCGAAAAACACCCTGTATCTGC
AAATGAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGCGC
GTGGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGAT
GTTTGGGGCCAAGGCACCCTGGTGACGGTTAGCTCA (SEQ ID NO: 1);

(b) a nucleic acid molecule which encodes a polypeptide having the amino acid sequence as shown in SEQ ID NO: 2:

QVELVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINA
SGTRTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKP
YGYVRYFDVWGQGTLVTVSS (SEQ ID NO: 2; "**N**" in bold representing the herein defined Asn on position 52 of the variable region of the heavy chain);

(c) a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) or (b) and which encodes a polypeptide which is capable of binding to the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 3)

or a fragment thereof which comprises at least 15 amino acids;

(d) a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) or (b) and which encodes a polypeptide which is capable of binding to at least two regions on the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 3)

or to at least two regions of a fragment of SEQ ID NO. 3 which comprises at least 15 amino acids

whereby said two regions on the β-A4 peptide Aβ4 or said fragment thereof comprise the amino acids on position 3 to 6 and on position 18 to 26 of SEQ ID No. 3; or

(e) a nucleic acid sequence that is degenerate to a nucleic acid sequence as defined in any one of (a) to (d).

The person skilled in the art is aware of the fact that the term "nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) or (b) and which encodes a polypeptide which is capable of binding to at least two regions on the β-A4 peptide/Aβ4" as employed herein relates to a coding strand of a double stranded nucleic acid molecule whereby the non-coding strand hybridizes to the above identified nucleic acid molecule of (a) and (b).

As pointed out above, the purified antibody molecule comprising the herein defined Asn-glycosylation may, inter alia, be characterized and described as an antibody molecule wherein the variable region comprising a glycosylated Asn is comprised in a heavy chain selected from the group consisting of:

(a) a heavy chain polypeptide encoded by a nucleic acid molecule as shown in SEQ ID NOS: 5, 23 or 25;

(b) a heavy chain polypeptide having the amino acid sequence as shown in SEQ ID NO: 6 or 26;

(c) a heavy chain polypeptide encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) and which encodes a polypeptide which is capable of binding to the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 3)

or a fragment thereof which comprises at least 15 amino acids; or

(d) a heavy chain polypeptide encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) and which encodes a polypeptide which is capable of binding to at least two regions on the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(SEQ ID NO: 3)

or to at least two regions of a fragment of SEQ ID NO. 3 which comprises at least 15 amino acids

whereby said two regions on the β-A4 peptide Aβ4 or said fragment thereof comprise the amino acids on position 3 to 6 and on position 18 to 26.

The above-identified antibody (e.g. an exemplified antibody of the invention) may also comprise an L-chain with the following amino acid sequence:

DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVP
ARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 22)

or an L-chain as, e.g. encoded by the following nucleic acid sequence:

gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcgagccagagcgtga
gcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatggcgcgagcagccgtgcaactggggtcc
cggcgcgttttagcggctctggatccggcacggattttaccctgaccattagcagcctggaacctgaagactttgcgacttattattgccttca
gatttataatatgcctattacctttggccagggtacgaaagttgaaattaaacgtacggtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg
ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggg
gagagtgttag (SEQ ID NO: 21)

As printed out above, the purified antibody molecule comprising the herein defined Asn-glycosylation in the heavy chain may further comprise a light chain selected from the group consisting of:

(a) a light chain polypeptide encoded by a nucleic acid molecule as shown in SEQ ID NOS: 7, 21, 24 or 27;

(b) a light chain polypeptide having the amino acid sequence as shown in SEQ ID NO: 8, 22 or 28;

(c) a light chain polypeptide encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) and which encodes a polypeptide which is capable of binding to the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(SEQ ID NO: 3)

or a fragment thereof which comprises at least 15 amino acids; or

(d) a light chain polypeptide encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of (a) and which encodes a polypeptide which is capable of binding to at least two regions on the β-A4 peptide/Aβ4 as shown in the following amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(SEQ ID NO: 3)

or to at least two regions of a fragment of SEQ ID NO. 3 which comprises at least 15 amino acids

The term "hybridization" or "hybridizes" as used herein in context of nucleic acid molecules/DNA sequences may relate to hybridizations under stringent or non-stringent conditions. If not further specified, the conditions are preferably non-stringent. Said hybridization conditions may be established according to conventional protocols described, for example, in Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001); Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989), or Higgins and Hames (Eds.) "Nucleic acid hybridization, a practical approach" IRL Press Oxford, Washington DC, (1985). The setting of conditions is well within the skill of the artisan and can be determined according to protocols described in the art. Thus, the detection of only specifically hybridizing sequences will usually

require stringent hybridization and washing conditions such as 0.1xSSC, 0.1% SDS at 65°C. Non-stringent hybridization conditions for the detection of homologous or not exactly complementary sequences may be set at 6xSSC, 1% SDS at 65°C. As is well known, the length of the probe and the composition of the nucleic acid to be determined constitute further parameters of the hybridization conditions. Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility. Hybridizing nucleic acid molecules also comprise fragments of the above described molecules. Such fragments may represent nucleic acid sequences which code for a non-functional antibody molecule or a non-functional fragment thereof or for a CDR as defined herein, and which have a length of at least 12 nucleotides, preferably at least 15, more preferably at least 18, more preferably of at least 21 nucleotides, more preferably at least 30 nucleotides, even more preferably at least 40 nucleotides and most preferably at least 60 nucleotides. Furthermore, nucleic acid molecules which hybridize with any of the aforementioned nucleic acid molecules also include complementary fragments, derivatives and allelic variants of these molecules. Additionally, a hybridization complex refers to a complex between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., Cot or Rot analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which, e.g., cells have been fixed). The terms complementary or complementarity refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between single-stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between

nucleic acids strands.

The term "hybridizing sequences" preferably refers to sequences which display a sequence identity of at least 40%, preferably at least 50%, more preferably at least 60%, even more preferably at least 70%, particularly preferred at least 80%, more particularly preferred at least 90%, even more particularly preferred at least 95% and most preferably at least 97% identity with a nucleic acid sequence as described above encoding an antibody molecule. Moreover, the term "hybridizing sequences" preferably refers to sequences encoding an antibody molecule having a sequence identity of at least 40%, preferably at least 50%, more preferably at least 60%, even more preferably at least 70%, particularly preferred at least 80%, more particularly preferred at least 90%, even more particularly preferred at least 95% and most preferably at least 97% identity with an amino acid sequence of the antibody molecule as described herein above.

In accordance with the present invention, the term "identical" or "percent identity" in the context of two or more nucleic acid or amino acid sequences, refers to two or more sequences or subsequences that are the same, or that have a specified percentage of amino acid residues or nucleotides that are the same (e.g., 60% or 65% identity, preferably, 70-95% identity, more preferably at least 95% identity), when compared and aligned for maximum correspondence over a window of comparison, or over a designated region as measured using a sequence comparison algorithm as known in the art, or by manual alignment and visual inspection. Sequences having, for example, 60% to 95% or greater sequence identity are considered to be substantially identical. Such a definition also applies to the complement of a test sequence. Preferably the described identity exists over a region that is at least about 15 to 25 amino acids or nucleotides in length, more preferably, over a region that is about 50 to 100 amino acids or nucleotides in length. Those having skill in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on CLUSTALW computer program (Thompson Nucl. Acids Res. 2 (1994), 4673-4680) or FASTDB (Brutlag Comp. App. Biosci. 6 (1990), 237-245), as known in the art.

Although the FASTDB algorithm typically does not consider internal non-matching deletions or additions in sequences, i.e., gaps, in its calculation, this can be corrected manually to avoid an overestimation of the % identity. CLUSTALW, however, does take sequence gaps into account in its identity calculations. Also available to those having skill in this art are the BLAST and BLAST 2.0 algorithms (Altschul, Nucl. Acids Res. 25 (1997), 3389-3402; Altschul, J. Mol.

Evol. 36 (1993), 290-300; Altschul, J. Mol. Biol. 215 (1990), 403-410). The BLASTN program for nucleic acid sequences uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, and an expectation (E) of 10. The BLOSUM62 scoring matrix (Henikoff Proc. Natl. Acad. Sci., USA, 89, (1989), 10915) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

Moreover, the present invention also relates to nucleic acid molecules the sequence of which is degenerate in comparison with the sequence of an above-described hybridizing molecule. When used in accordance with the present invention the term "being degenerate as a result of the genetic code" means that due to the redundancy of the genetic code different nucleotide sequences code for the same amino acid.

In order to determine whether an amino acid residue or nucleotide residue in a given antibody sequence corresponds to a certain position in the amino acid sequence or nucleotide sequence of any of e.g. SEQ ID NOS: 1, 5, 23 and 25, the skilled person can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as those mentioned further down below in connection with the definition of the term "hybridization" and degrees of homology.

For example, BLAST 2.0, which stands for Basic Local Alignment Search Tool BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.), can be used to search for local sequence alignments. BLAST, as discussed above, produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying similar sequences. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP). An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any

database sequence whose match satisfies E is reported in the program output.

Analogous computer techniques using BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.) are used to search for identical or related molecules in nucleotide databases such as GenBank or EMBL. This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score which is defined as:

$$\frac{\%\text{sequence identity} \times \%\text{ maximum BLAST score}}{100}$$

and it takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules. Another example for a program capable of generating sequence alignments is the CLUSTALW computer program (Thompson, Nucl. Acids Res. 2 (1994), 4673-4680) or FASTDB (Brutlag Comp. App. Biosci. 6 (1990), 237-245), as known in the art.

In one embodiment the present invention provides glycosylated ANTIBODY isoforms wherein the glycosylation on Asn in the V_H region is selected from the group consisting of

(a) a sugar structure of the biantennary complex type without core fucosylation;
(b) a sugar structures of the biantennary hybrid type;
(c) a sugar structures of the biantennary oligomannose type; and
(d) a bi-antennary structure of any of the structures as provided in appended figure 5 or appended figure 27.

The corresponding sugar structure does, in one embodiment of the antibody/antibodies of this invention not comprise a core fucosylation.

The corresponding N-glycosylation may predominantly consist of sugar structures of the biantennary complex type (≥ 75%; mainly 80 – 90%) without core fucosylation and highly sialidated with up to 80% of antennae. Minor sugar structures belong to the biantennary hybrid and the oligomannose type (≤ 25%), respectively and are also shown in appended Figures 5 and

27. The glycosylation structures in the variable region are resistant to cleavage by N-glycosidase F from the protein (amino acid polypeptide).

In one embodiment the dominant complex biantennary sugar structures are further characterized

- by containing one or two sialic acids attached to either the one or the other antenna or to both antennae. The sialic acid is of the N-acetyl neuraminic acid type and is most likely bound in alpha 2,3 linkage to the terminal beta 1,4 linked galactoses.
- by lacking core fucosylation, i.e. lacking the fucose residue attached in alpha 1,6 linkage to the innermost N-acetyl-glucosamine at the reducing end of the sugar chain.

In one embodiment the hybrid sugar structures are further characterized

- by containing a complex type antenna (a lactosaminyl unit (GlcNAc-Gal) attached to the core sugar structure) as one arm of the bi-antennary structure. This arm predominantly contains N-acetyl neuraminic acid attached to the terminal beta 1,4 linked galactose.
- by having one up to 3 additional mannose subunits attached to the core sugar structure as the other antenna.
- by lacking core fucosylation, i.e. lacking the fucose residue attached in alpha 1,6 linkage to the innermost N-acetyl-glucosamine at the reducing end of the sugar chain.

In one embodiment the oligomannose type sugar structures are further characterized

- by containing 4 (Man4 →GlcNAc2), 5 (Man5→GlcNAc2) or 6 (Man6→GlcNAc2) mannose subunits in the complete sugar structure, i.e. including the 3 branching mannose subunits present in a typical N-linked core sugar structure.
- by lacking core fucosylation, i.e. lacking the fucose residue attached in alpha 1→6 linkage to the innermost N-acetyl-glucosamine at the reducing end of the sugar chain.

In another embodiment of the present invention, a composition is provided which comprises an antibody molecule being characterized in that one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H) and an antibody molecule being characterized in that two antigen binding sites comprise a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H), i.e. a composition comprising mono-glycosylated ANTIBODY and double-glycosylated ANTIBODY, and is hereinafter referred to as

ANTIBODY COMPOSITION. The term ANTIBODY COMPOSITION also relates to compositions which comprise molecules comprising at least one glycosylated V_H region as defined herein or at least one glycosylated CDR of said V_H region, whereby said molecules may, inter alia be immunoglobulins or immunoglobulin isoforms and modifications as described above. For example said composition may also comprise single chain antibodies (scFvs) or bispecific molecules comprising glycosylated, V_H-derived CDR regions.
Further definitions of the ANTIBODY COMPOSITION of this invention are provided below.

The ANTIBODY COMPOSITION does not or does merely to a very low extent comprise "in V_H non-glycosylated" antibody molecules, i.e. antibodies that do not comprise the herein defined glycosylation in the variable region, in particular the variable part of the heavy chain (V_H).

In context of this invention and in particular in context of the antibody mixtures provided herein, the term "does not or does merely to a very low extent comprise non-glycosylated antibody molecules" means that the ANTIBODY COMPOSITION comprises less than 10%, e.g. less than 5%, for example less than 4%, for example less than 3%, for example less than 2%, for example less than 1%, for example less than 0.5 or less of the/a non-glycosylated isoform as described herein.

Accordingly, in one embodiment, the present invention provides for an antibody preparation comprising mono-glycosylated and/or double-glycosylated antibodies (said glycosylation being located in the variable region of the heavy chain) and being devoid of antibody molecules without glycosylation in the variable region.

Again, the term "devoid of antibody molecules without glycosylation in the variable region" relates to antibody preparations/antibody mixtures/antibody pools which comprise at the most 10%, e.g. at the most 5%, e.g. at the most 4%, e.g. at the most 3%, e.g. at the most 2%, e.g. at the most 1%, e.g. at the most 0.5%, e.g. at the most 4%, e.g. at the most 3%, e.g. at the most 2%, e.g. at the most 1%, e.g. at the most 0.5%, e.g. at the most 0.3%, e.g. at the most 0.2% non-glycosylated isoforms as described herein.

In one embodiment the present invention provides a composition which does not comprise more than 0.5 % antibody isoforms which are non-glycosylated in their variable regions, e.g. are non-glycosylated in the variable region of the heavy chain.

As pointed out above, in one embodiment of the present invention, a mixture of mono- and double-glycosylated antibodies, e.g. immunoglobulins, is provided, said mixture being devoid of antibody molecules without glycosylation in the variable region. Antibodies devoid of such a post-translational modification in the variable region, e.g. in both variable regions of the heavy chain (both (V_H)-regions) is, in context of this invention considered as an "non-glycosylated form", comprising no glycosylation in the variable region of the heavy chain. Yet, this "non-glycosylated form" may nevertheless comprise (a) glycosylation(s) in the constant region (C-region) of the antibody, for example, and most commonly at the well conserved glycosylation site of the Fc-part, in particular the asparagine (Asn) 306 in the non-variable/constant Fc-part as defined herein.

The glycosylated ANTIBODY isoforms on their own or as a combination of mono-glycosylated and double-glycosylated isoforms are very useful and advantageous therapeutic antibody preparations for the treatment of Alzheimer Disease (AD), and other amyloid related disorders like Down's syndrome, Hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, ALS (amyotrophic lateral sclerosis), Creutzfeld Jacob disease, HIV-related dementia and motor neuropathy. The glycosylated ANTIBODY isoforms on their own or as a combination of mono-glycosylated and double-glycosylated isoforms are also unique diagnostic tools.

Both glycosylated isoforms as described herein show improved and highly effective brain penetration **in vivo**. Effective brain penetration and specific binding to amyloid-β plaques can be demonstrated in PS2APP mice, a mouse model for AD-related amyloidosis.

Furthermore, improved specificity for genuine human amyloid-β plaques by immunohistochemical stainings in vitro with significantly reduced unspecific stickiness could be detected. The minimal effective concentration for consistent staining of human amyloid-β plaques was determined to be 10 ng/ml, as documented in the appended examples.

As documented in the appended examples, the separation and characterization of differently glycosylated antibodies, e.g. immunoglobulins revealed that the glycosylation of the variable region of the heavy chain has a surprising influence on the antigen binding to Aβ peptides, the diagnostic value, the pharmacological profile and functional activity. The purified antibody molecules may be submitted to MS-analytics, binding studies (Biacore) and epitope mapping

(Pepspot analysis) binding to soluble Aβ, dissociation of aggregated Aβ and microscopical analysis of binding to β-amyloid plaques *in vivo* and *in vitro*.

In one embodiment of the present invention, the purified ANTIBODY or the ANTIBODY COMPOSITION is capable of specifically recognizing the β-A4 peptide/Aβ4.

Accordingly, and as described herein, purified ANTIBODY or ANTIBODY COMPOSITION relates in a specific embodiment to ANTIBODY or ANTIBODY COMPOSITION capable of specifically recognizing two regions (the N-terminal region and the central/middle part) of Aβ/Aβ4.

The term "specifically recognizing" means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two amino acids of each of the two regions of β-A4 as defined herein. Said term relates to the specificity of the antibody molecule, i.e. to its ability to discriminate between the specific regions of the β-A4 peptide as defined herein and another, not related region of the β-A4 peptide or another, not APP-related protein/peptide/(unrelated) tests-peptide. Accordingly, specificity can be determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. Such methods also comprise the determination of K_D-values as, inter alia, illustrated in the appended examples. The peptide scan (pepspot assay) is routinely employed to map linear epitopes in a polypeptide antigen. The primary sequence of the polypeptide is synthesized successively on activated cellulose with peptides overlapping one another. The recognition of certain peptides by the antibody to be tested for its ability to detect or recognize a specific antigen/epitope is scored by routine colour development (secondary antibody with an enzyme or a dye, like horseradish peroxidase or 4-chloronaphthol or hydrogenperoxide), by a chemoluminescence reaction or similar means known in the art. In the case of, inter alia, chemoluminescence reactions or the use of a secondary fluorescent antibody, the reaction can be quantified. If the antibody reacts with a certain set of overlapping peptides one can deduce the minimum sequence of amino acids that is necessary for reaction; see illustrative examples provided in accordance with this invention.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5569

The same assay can reveal two distant clusters of reactive peptides, which indicate the recognition of a discontinuous, i.e. conformational epitope in the antigenic polypeptide (Geysen (1986), Mol. Immunol. 23, 709-715).

In addition to the pepspot assay, standard ELISA assay can be carried out. As demonstrated in the appended examples small hexapeptides may be coupled to a protein and coated to an immunoplate and reacted with antibodies to be tested. The scoring may be carried out by standard colour development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogenperoxide). The reaction in certain wells is scored by the optical density, for example at 450 nm. Typical background (=negative reaction) may be 0.1 OD, typical positive reaction may be 1 OD. This means the difference (ratio) positive/negative can be more than 10 fold. Further details are given in the appended examples. Additional, quantitative methods for determining the specificity and the ability of "specifically recognizing" the herein defined two regions of the β-A4 peptide are given herein below.

The term "two regions of the β-A4 peptide" relates to two regions relating e.g. to the N-terminal amino acids 3 to 6 and a central/middle epitope on position amino acids 18 to 24 of SEQ ID No. 3 (the β-A4 peptide). As documented in the appended examples, in particular the double-glycosylated ANTIBODY A isoform provided and exemplified herein (see appended examples) detects two parts of the Aß molecule, the first part comprising amino acid 1 to 10 in the N-terminus and the second part comprising amino acids 17 to 26 of the central/middle part of Aß (as shown in SEQ ID No. 3). Accordingly, in the antibody mixtures provided herein and comprising the mono- as well as the double-glycosylated isoforms of the antibodies as provided herein, the two regions may also somewhat broadened, then comprising, e.g. amino acids 1 to 10 (or to 11 or to 12) or a shorter part thereof and amino acids 17 to 26 (or amino acids 16 to 27) or a shorter part comprised between amino acids 17 to 26, like e.g. amino acids 19 to 26 or 20 to 26). The term "β-A4 peptide" in context of this invention relates to the herein above described Aβ39, Aβ41, Aβ43, in particular to Aβ40 and Aβ42. Aβ42 is also depicted in appended SEQ ID NO: 3. It is of note that the term "two regions of the β-A4 peptide" also relates to an "epitope" and/or an "antigenic determinant" which comprises the herein defined two regions of the β-A4 peptide or parts thereof. In accordance with this invention, said two regions of the β-A4 peptide are separated (on the level of the amino acid sequence) in the primary structure of the β-A4 peptide by at least one amino acid, e.g. by at least two amino acids, e.g. by at least three amino

acids, e.g. by at least four amino acids, e.g. by at least five amino acids, e.g. by at least six amino acids. As shown herein and as documented in the appended examples, the inventive antibodies/antibody molecules detect/interact with and/or bind to two regions of the β-A4 peptide as defined herein, whereby said two regions are separated (on the primary structure level of the amino acid sequence) by at least one amino acid and wherein the sequence separating said two regions/"epitope" may comprise more then seven, amino acids, more than 8 amino acids, more than 10 amino acids or even about 14 amino acids.

The term "two regions of the β-A4 peptide" may also relate to a conformational epitope or a discontinuous epitope consisting of said two regions or parts thereof; see also Geysen (1986), loc. cit. In context of this invention, a conformational epitope is defined by two or more discrete amino acid sequences separated in the primary sequence which come together on the surface when the polypeptide folds to the native protein (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6). The antibody molecules of the present invention are envisaged to specifically bind to/interact with a conformational epitope(s) composed of and/or comprising the two regions of β-A4 described herein or parts thereof as disclosed herein below. The "antibody molecules" of the present invention are thought to comprise a simultaneous and independent dual specificity to (a) an amino acid stretch comprising amino acids 1 to 11 (or (a) part(s) thereof) of β-A4 and (b) an amino acid stretch comprising amino acids 16 to 27 (or (a) part(s) thereof) of β-A4 (SEQ ID NO. 3). Fragments or parts of these stretches comprise at least two, in most cases at least three amino acids.

Antibody molecules, e.g. immunoglobulins could, inter alia, be expressed in three systems: a) in transiently transfected human embryonic kidney cells containing the Epstein barr virus nuclear antigen (HEK 293 EBNA, Invitrogen), b) in transiently transfected Chinese hamster ovary cells (CHO), and c) in stably transfected CHO cell lines (CHO K1 and CHO K1 SV, Lonza Biologics). The three different antibody molecules (non-, mono or double-glycosylated) may be separated by specific purification steps, comprising protein A purification, cation exchange chromatography as well as size column separation as detailed below.

In one embodiment of the invention, the antibody molecule is recombinantly produced, e.g. in a CHO-cell or in a HEK 293 cell, preferably CHO-cells. In a particular embodiment the above identified glycosylation patterns may be obtained after expression in CHO-cells. CHO-cells are very well known in the art and comprise, inter alia, the CHO-cells as employed in the

experimental part, like CHO K1 or CHO K1 SV cells. Commonly used HEK 293 cells are HEK 293 EBNA.

The recombinant expression of the glycosylated, inventive antibody is carried out, as shown in the examples in a eukaryotic expression system in particular in CHO-cells. However, further expression cells, i.e. eukaryotic cells may be envisaged. Eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, e.g. those of the genus Saccharomyces, e.g. those of the species Saccharomyces cerevisiae. Suitable animal cells are, for instance, insect cells, vertebrate cells, e.g. mammalian cells, such as e.g. NSO, MDCK, U2-OSHela, NIH3T3, MOLT-4, Jurkat, PC-12, PC-3, IMR, NT2N, Sk-n-sh, CaSki, C33A. Also human cell lines are envisaged. These host cells, e.g. CHO-cells, provide post-translational modifications to the antibody molecules of the invention, including leader peptide or signal sequence removal, folding and assembly of H (heavy) and L (light) chains and most importantly glycosylation of the molecule at correct sides, namely in the variable region of the heavy chain. Such signal peptide or leader sequence is proteolytically cleaved by the host signalpeptidase during the secretory pathway during its recombinant production e.g. in CHO cells. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC). In accordance with the present invention, it is furthermore envisaged that primary cells/cell cultures may function as host cells. Said cells are in particular derived from insects (like insects of the species Drosophila or Blatta) or mammals (like human, swine, mouse or rat). Said host cells may also comprise cells from and/or derived from cell lines like neuroblastoma cell lines.

Accordingly, the antibody molecule of the invention is prepared using a recombinant expression system. An example for such system, as pointed out above, is a mammalian expression system using Chinese hamster ovary (CHO) cells. These may be used with the glutamine synthetase (GS) system (WO 87/04462; WO 89/01036; Bebbington, 1992, Biotechnology (N Y), 10, 169-75). This system involves the transfection of a CHO cell with a gene encoding the GS enzyme and the desired antibody genes. CHO cells are then selected which grow in glutamine free media and are also subjected to inhibition of the GS enzyme using methionine sulphoximine (MSX). In order to survive, the cells will amplify the GS enzyme expression and concomitantly the expression of the mAb.

Another possible expression system is the CHO dhfr- system, where the CHO cells are deficient for dihydrofolate reductase (dhfr-) and dependent on thymidine and hypoxanthine for growth. The parenteral CHO dhfr- cell line is transfected with the antibody and the dhfr gene thus enabling the selection of CHO cell transformants of the dhfr+ phenotype. Selection is carried out in the absence of thymidine and hypoxanthine. Expression of the antibody gene may be increased by amplification using methotrexate (MTX). This drug is a direct inhibitor of the dhfr enzyme and allows for isolation of resistant colonies which amplify their dhfr gene copy number and therefore the antibody gene sufficiently to survive under these conditions.

Purified antibody molecules, e.g. immunoglobulins, may be prepared by a method comprising the steps of

(a) recombinantly expressing a heterologous nucleic acid molecule encoding an antibody molecule as defined herein above in a mammalian cell, e.g. a CHO or a HEK 293 cell; and

(b) purifying said recombinantly expressed antibody molecule by a method comprising the steps of

  (b1) protein A column purification;

  (b2) ion exchange column purification, e.g. a cation exchange chromatography; and, optionally,

  (b3) size exclusion column purification.

The purification protocol may comprise further steps, like further concentration steps, e.g. diafiltration or analytical steps, e.g. involving analytical columns. The method/process may also comprise virus inactivation steps and/or viral removal steps e.g. via filtrations/nano-filtrations. It is also envisaged and feasible that particular certain steps are repeated (e.g. two ion exchange chromatography steps may be carried out) or that certain steps (e.g. size exclusion chromatography) may be omitted.

Protein A is a group specific ligand which binds to the Fc region of most IgG1 isotypes. It is synthesized by some strains of Staphylococcus aureus and can be isolated therefrom and coupled to chromatographic beads. Several types of gel preparations are available commercially.

An example for a protein A column which may be used is a MabSelect (Trademark) column. Ideally the column is equilibrated with 25 mM Tris/HCl, 25 mM NaCl, 5 mM EDTA, the cell

culture supernatant is loaded onto the column, the column is washed with 1 M Tris/HCl pH 7,2 and the antibody is eluted at pH 3.2 using 100 mM acetic acid.

Cation-exchange chromatography exploits interactions between positively charged groups in a stationary phase and the sample which is in the mobile phase. When a weak cation exchanger (e.g. CM Toyopearl 650®) is used, the following chromatographic steps are performed: After preequilibration with 100 mM acetic acid pH 4, loading of Protein A eluate and washing with 100 mM acetic acid pH 4 the antibody is eluted and fractionated by applying steps of 250 mM sodium acetate (pH 7.8-8.5) and 500 mM sodium acetate (pH 7.8-8.5). With the first step a mixture of double-glycosylated isoform fraction and mono-glycosylated isoform fraction are normally eluted, using the second step the non-glycosylated isoform fraction is normally eluted.

From a strong cation exchanger (e.g. SP Toyopearl 650) the antibody can be eluted by salt steps: After equilibration of the column with 50 mM acetic acid pH 5.0, loading the Protein A eluate with pH 4 the first elution step using 50 mM acetic acid and 210 mM sodium chloride is performed. Then a second elution step of 50 mM acetic acid and 350 mM sodium chloride is applied. By the first salt step a mixture of the double-glycosylated isoform fraction and mono-glycosylated isoform fraction are normally eluted, by the second salt step the non-glycosylated isoform is normally eluted.

In addition the antibody may also be eluted from a strong cation exchanger column (e.g. SP-Sepharose®) by a salt gradient: After preequilibration, loading and washing the column at pH 4.5 a salt gradient is applied from 50 mM MES pH 5.8 to 50 mM MES /1 M sodium chloride pH 5.8. Here the double-glycosylated isoform , mono-glycosylated isoform and non-glycosylated isoform fractions are normally eluted separately. In the following double-glycosylated isoform fraction and mono-glycosylated isoform fraction may be pooled to result in the product pool and/or a desired antibody mixture.

Further purification of the mixture of double- and mono-glycosylated antibody molecules, e.g. immunoglobulins, may be performed by size exclusion chromatography. An example of a useful column is a Superdex 200® column. Examples of running buffers include histidine/sodium chloride, e.g. 10 mM histidine/125 mM sodium chloride/pH 6, and phosphate buffered saline (PBS).

Anion exchange chromatography in the flow through mode followed by a concentration/ diafiltration is an alternative purification step. Q Sepharose® is an example for a resin for the anion exchange step. For example, the eluate from the SP chromatography may be threefold diluted with 37,5 mM Tris/HCl pH 7.9 and passed over a Q-Sepharose column pre-equilibrated with 25 mM Tris/83 mM sodium acetate. The flow through is collected, adjusted to pH 5.5 and concentrated by ultrafiltration using e.g. a Hydrosart 30 kD® membrane. In the following the concentrate may be diafiltrated against for example 10 volumes of 20 mM histidine/HCl pH 5.5.

The above recited purification protocol may also comprise as an additional step (c) an analytical chromatography step, like the use of a Mono-S HR5/5 column. However, also further steps, like diafiltration, for example for concentration of the antibody molecules, is envisaged.

In one embodiment of the present invention, a composition, antibody preparation or antibody pool is provided comprising antibody molecules as described herein or antibody molecules as prepared by the method provided above. In this embodiment of the invention, said composition comprises mono- or double-glycosylated antibodies. In another embodiment, said composition comprises mono- and double-glycosylated (in the variable region(s) of the heavy chain(s)) antibodies and said composition is derived of antibody molecules which lack the glycosylation in the variable region. In context of this embodiment, the term "antibody pool" relates to a mixture of mono- and double-glycosylated (in the variable region(s) of the heavy chain(s)) antibodies which may have been individually isolated and then are combined to one mixture. The antibody mixtures or antibody pools provided herein may comprise 50% mono-glycosylated and 50% double-glycosylated antibodies as defined herein. However, also envisaged are the ratios of 30/70 to 70/30. Yet, the person skilled in the art is aware that also other ratios are envisaged in the antibody mixtures of this invention. For example, also 10/90 or 90/10, 20/80 or 80/20 as well as 40/60 or 60/40 may be employed in context of this invention. As also documented in the examples, a particular useful ratio in the ANTIBODY MIXTURES of the invention comprises double-glycosylated and mono-glycosylated antibody as defined herein above is a ratio from 40/60 to 45/55.

The compositions provided herein are particularly useful in diagnostic or in a pharmaceutical composition.

Accordingly, the invention provides for diagnostic or pharmaceutical compositions comprising

(a) an antibody molecule as defined above comprising one antigen binding site with a

glycosylated Asn;

(b) an antibody molecule as defined above, comprising two antigen binding sites with a glycosylated Asn; or, most preferably,

(c) a combination of antibody molecules (a) to (b).

The combination (c) as provided herein, comprising the antibody molecule(s) comprising one antigen binding site with a glycosylated Asn and the antibody molecule(s) comprising two antigen binding sites with a glycosylated Asn are devoid of non-glycosylated (in respect to the variable region of the heavy chain) isoforms. As pointed out above, the term "devoid of non-glycosylated (in respect to the variable region of the heavy chain) isoform" relates to combinations/antibody pools/antibody preparations, wherein less than 5%, e.g. less than 4%, less than 3%, less than 2%, less than 1% or even less than 0.5% of the antibody species in said combination is non-glycosylated in the variable region of the heavy chain. As demonstrated in the examples, said combinations/antibody pools/antibody preparations may comprise almost no (less than 0.5 %) non-glycosylated isoforms. The percentage and/or the amount of a given glycosylation isoform (as defined herein, e.g. glycosylation in the variable region of the heavy chain, see inter alia appended figure 14) in a given ANTIBODY COMPOSITION may easily be determined by methods known in the art. These methods comprise, but are not limited to, mass spectrometry, SDS-PAGE analysis ion exchange, HPLL, ELISA and the like.

As shown in the appended examples, the specific and sensitive immuno-decoration of genuine Alzheimer's β-amyloid plaques by the antibodies of the invention is demonstrated *in vitro* with immunohistochemical staining experiments using cryo-sections of human brain tissue from AD patients. Effective staining of β-amyloid plaques from brain slices was demonstrated also with human anti-Aβ antibodies from patients vaccinated with Aβ (Hock, 2002, Nature Medicine, 8, 1270-1275). Further, immuno-decoration is also demonstrated in a transgenic animal model featuring human β-amyloid plaque burden (Richards, 2003, J. Neuroscience, 23, 8989-9003). In similar animal models it had been demonstrated that this plaque binding led to their clearance and subsequently to an improvement of disease related symptoms, whereas the involvement of Fc-dependent processes had been discussed (Bard, 2000, Nature Medicine, 6, 916-919; Wilcock, 2003, Neurobiology Disease, 15, 11-20; Wilcock, 2004, J. Neuroscience, 24, 6144-6151). Furthermore, effective binding of anti-Aβ antibodies to β-amyloid plaques was reported to correlate with slower disease progression (Hock, 2002, Nature Medicine, 8, 1270-1275; Hock,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5576

2003, Neuron, 38, 547-554). This and post-mortem analysis of human brain tissue suggests that phagocytosis of microglia cells is mechanistically involved in the plaque clearance in man (Nicoll, 2003, Nature Medicine, 9, 448-452). Therefore the antibody of the present invention or comprised in particular in pharmaceutical compositions is a human IgG1, which is mainly responsible for FcR-dependent processes in humans. The efficient β-amyloid plaque immuno-decoration of the antibodies of the invention/the mixture of the invention suggests that the drug will be efficacious for passive immunization to clear existing and prevent formation of β-amyloid plaques in humans.

In addition antibodies should preferably cross the blood-brain-barrier to reach their place of destination. For large size molecules as human IgGs this process is dramatically reduced, so that only about 0.1 to 0.2% of the plasma concentration of an antibody can be reached in CSF. The mechanism of plaque clearance is still a subject of controversial debates, which might involve peripheral effects on the Aβ peptide (Dodart, 2002, Nature Neuroscience, 5: 452-457). Thus, the generated therapeutic antibody or the corresponding inventive mixtures of mono- and double glycosylated (in the variable region heavy chain) of the invention have also the property to depolymerize Aβ multimers *in vitro* without involvement of Fc-dependent processes and to bind to soluble Aβ monomers and oligomers in CSF, since neutralization of soluble monomeric Aβ peptides or oligomeric Aβ peptides (e.g. aggregation intermediates) may also contribute to overall amyloid lowering effect (Du, 2003, Brain, 126: 1-5).

The compositions of the invention may be administered in solid or liquid form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). Said composition may comprise on or more antibodies/antibody molecules of the invention most preferably a mixture of mono- and double-glycosylated antibodies as provided herein.

It is preferred that said pharmaceutical composition, optionally comprises a pharmaceutically acceptable carrier and/or diluent. The herein disclosed pharmaceutical composition may be particularly useful for the treatment of neurological and/or neurodegenerative disorders. Said disorders comprise, but are not limited to Alzheimer's disease, amyothrophic lateral sclerosis (ALS), hereditary cerebral hemorrhage with amyloidosis Dutch type, Down's syndrome, HIV-dementia, Parkinson's disease and neuronal disorders related to aging. The pharmaceutical composition of the invention is, inter alia, envisaged as potent inhibitor of amyloid plaque formation or as a potent stimulator for the de-polymerization of amyloid plaques. Therefore, the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5577

present invention provides for pharmaceutical compositions comprising the compounds of the invention to be used for the treatment of amyloidogenic diseases/disorders. The term "amyloidogenic disease/disorder" includes any disease associated with or caused by the formation or deposition of amyloid fibrils and/or pathological APP proteolysis. Exemplary amyloidogenic disease include, but are not limited to Alzheimer's disease (AD), Down's Syndrome, dementia associated with Lewy body formation, Parkinson's Disease with dementia, mild cognitive impairment, cerebral amyloid angiopathy and vascular dementia. Different amyloidogenic diseases are defined and/or characterized by the nature of the polypeptide-component of the amyloid deposits. For example, the amyloid-β protein is characteristic for the amyloid deposits found in subjects having Alzheimer's disease.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. Suitable carriers may comprise any material which, when combined with the anti-Aβ specific binding agent or antibody, retains the high-affinity binding of Aβ and is nonreactive with the subject's immune systems including excipients, surfactants, tonicity agents and the like; see Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by parenteral, subcutaneous, intraperitoneal, topical, intrabronchial, intrapulmonary and intranasal administration and, if desired for local treatment, intralesional administration. Parenteral administrations include intraperitoneal, intramuscular, intradermal, subcutaneous intravenous or intraarterial, administration. It is particularly preferred that said administration is carried out by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an external or internal target site, like the brain.

Pharmaceutical compositions comprising the herein described glycosylated antibodies are prepared by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5578

preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-Methylglucosamine (so-called "Meglumine"), galactosamine and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X or polyethylene glycol (PEG).

The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization), however also solutions comprising antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

Exemplary antibody concentrations in the pharmaceutical composition may range from about 1 mg/mL to about 200 mg/ml or from about 50 mg/mL to about 200 mg/mL, or from about 150 mg/mL to about 200 mg/mL. For clarity reasons, it is emphasized that the concentrations as indicated herein relate to the concentration in a liquid or in a liquid that is accurately reconstituted from a solid form.

An aqueous formulation of the antibody may be prepared in a pH-buffered solution, e.g., at pH ranging from about 4.0 to about 7.0, or from about 5.0 to about 6.0, or alternatively about 5.5. Examples of buffers that are suitable for a pH within this range include phosphate-, histidine-, citrate-, succinate-, acetate-buffers and other organic acid buffers. The buffer concentration can be from about 1 mM to about 100 mM, or from about 5 mM to about 50 mM, depending, e.g., on the buffer and the desired tonicity of the formulation.

A tonicity agent may be included in the antibody formulation to modulate the tonicity of the formulation. Exemplary tonicity agents include sodium chloride, potassium chloride, glycerin and any component from the group of amino acids, sugars as well as combinations thereof.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5579

Preferably the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as physiological salt solution and the blood serum. Tonicity agents may be used in an amount of about 5 mM to about 350 mM, in particular in an amount of 105 mM to 305 nM.

A surfactant may also be added to the antibody formulation to reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic)., and sodium dodecyl sulphate (SDS). Preferred polyoxyethylenesorbitan-fatty acid esters are polysorbate 20,(sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). Preferred polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Preferred Polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Exemplary concentrations of surfactant may range from about 0.001% to about 1% w/v.

A lyoprotectant may also be added in order to protect the labile active ingredient (e.g. a protein) against destabilizing conditions during the lyophilization process. For example, known lyoprotectants include sugars (including glucose and sucrose); polyols (including mannitol, sorbitol and glycerol); and amino acids (including alanine, glycine and glutamic acid). Lyoprotectants are generally used in an amount of about 10 mM to 500 nM.

In one embodiment, the formulation contains the above-identified agents (i.e. glycosylated antibody, surfactant, buffer, stabilizer and/or tonicity agent) and is essentially free of one or more preservatives, such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, and combinations thereof. In another embodiment, a preservative may be included in the formulation, e.g., at concentrations ranging from about 0.001 to about 2 %(w/v).

In one embodiment, the antibody formulation of the invention is a liquid or lyophilized formulation suitable for parenteral administration that may comprise:

- about 1 to about 200 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,

- about 0.001 to about 1% of at least one surfactant;
- about 1 to about 100 mM of a buffer;
- optionally about 10 to about 500 mM of a stabilizer and/or about 5 to about 305 mM of a tonicity agent;
- at a pH of about 4.0 to about 7.0.

In a preferred embodiment, the parenteral formulation of the invention is a liquid or lyophilized formulation comprising:

- about 1 to about 200 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.04% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Sucrose,
- at pH 5.5.

In a more preferred embodiment, the parenteral formulation according to the invention also comprises a lyophilized formulation comprising:

- 15 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.04% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Sucrose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.04% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Sucrose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,

- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Sucrose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.04% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Trehalose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Trehalose,
- at pH 5.5

In another more preferred embodiment, the parenteral formulation according to the invention also comprises a liquid formulation comprising:

- 7.5 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.022% Tween 20 w/v,
- 120 mM L-histidine,
- 250 125 mM Sucrose,
- at pH 5.5;

or

- 37.5 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 10 mM L-histidine,
- 125 mM Sucrose,

- at pH 5.5;

or

- 37.5 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.01% Tween 20 w/v,
- 10 mM L-histidine,
- 125 mM Sucrose,
- at pH 5.5;

or

- 37.5 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 10 mM L-histidine,
- 125 mM Trehalose,
- at pH 5.5;

or

- 37.5 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.01% Tween 20 w/v,
- 10 mM L-histidine,
- 125 mM Trehalose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Trehalose,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,

- 20 mM L-histidine,
- 250 mM Mannitol,
- at pH 5.5;

or

- 75 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 140 mM Sodium chloride,
- at pH 5.5;

or

- 150 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Trehalose,
- at pH 5.5.

or

- 150 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Mannitol,
- at pH 5.5.

or

- 150 mg/mL of the herein described glycosylated antibodies or ANTIBODY COMPOSITION,
- 0.02% Tween 20 w/v,
- 20 mM L-histidine,
- 140 mM Sodium chloride,
- at pH 5.5.

or

- 10 mg/mL Abeta antibody ,

- 0.01% Tween 20 w/v,
- 20 mM L-histidine,
- 140 mM Sodium chloride,

at pH 5.5.

In one embodiment, the pharmaceutical composition of the present invention is the liquid formulation which comprises:

- 10 mg/mL Abeta antibody ,
- 0.01% Tween 20 w/v,
- 20 mM L-histidine,
- 140 mM Sodium chloride,

at pH 5.5.

In anther embodiment, the pharmaceutical composition of the present invention is the lyophilized formulation which comprises:

- 75 mg/mL Abeta antibody ,
- 0.04% Tween 20 w/v,
- 20 mM L-histidine,
- 250 mM Sucrose,

at pH 5.5.

The term "herein described glycosylated antibodies" in context of exemplified formulations may comprise in this invention the herein defined mono-glycosylated antibodies, the herein defined double-glycosylated antibodies as well as mixtures thereof.

The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 20 mg/kg body weight per dose, e.g. between 0.1 mg to 10 mg/kg body weight, e.g. between 0.5 mg to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned,

especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1 μg to 10 mg per kilogram of body weight per minute.

The pharmaceutical compositions as described herein may be formulated to be short-acting, fast-releasing, long-acting, or sustained-releasing. Hence, the pharmaceutical compositions may also be suitable for slow release or for controlled release.

Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-hydroxybutyric acid. Possible loss of biological activity and possible changes in immunogenicity of antibodies comprised in sustained-release preparations may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

Progress can be monitored by periodic assessment. The compositions, i.e. the mono- and/or double-glycosylated antibodies of the invention or a mixture thereof, may be administered locally or systemically. It is of note that peripherally administered antibodies can enter the central nervous system, see, inter alia, Bard (2000), Nature Med. 6, 916-919. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition. Said agents may be drugs acting on the central nervous system, like, neuroprotective factors, cholinesterase inhibitors, agonists of M1 muscarinic receptor, hormones, antioxidants, inhibitors of inflammation etc. It is particularly

preferred that said pharmaceutical composition comprises further agents like, e.g. neurotransmitters and/or substitution molecules for neurotransmitters, vitamin E or alpha-lipoic acid.

The person skilled in the art, in particular but not limiting biochemists, biologists, chemists, pharmacists and groups of said professionals are readily in a position to work and generate the above recited pharmaceutical compositions. Also medical personal skilled in they art, like attending physicians are aware how such pharmaceutical compositions may be administered to a patient in need of a treatment with the herein defined pharmaceutical compositions. Such an administration may comprise systemic administration, e.g. via infusions and/or injections. However, also the direct administration of the compounds and/or compound mixtures of the invention to the brain is envisaged. For example, the compound or compound mixture or compound formulation may be administered by direct intraventricular or intrathecal injection to the brain, preferably via slow infusion to minimize impact on brain parenchyma. Also slow release implant in the brain may be employed. It is also envisaged that gene therapy approaches are employed, for example by use of implanted recombinant cells that produce the antibodies as defined in this invention. These "recombinant cells" should be capable of providing the herein defined glycosylations in the variable regions/parts of the antibodies described herein, in particular the anti-Aβ antibodies of the invention. Yet, as pointed out above one advantage of the antibodies/antibody mixtures of the present invention is their capability to cross the blood-brain barrier and to bind to amyloid plaques.The pharmaceutical compositions of the invention described infra can be used for the treatment of all kinds of diseases hitherto unknown or being related to or dependent on pathological APP aggregation or pathological APP processing. They may be particularly useful for the treatment of Alzheimer's disease and other diseases where extracellular deposits of amyloid-β, appear to play a role. They may be desirably employed in humans, although animal treatment is also encompassed by the methods, uses and compositions described herein.

In a preferred embodiment of the invention, the composition of the present invention as disclosed herein above is a diagnostic composition further comprising, optionally, suitable means for detection. The diagnostic composition comprises at least one of the aforementioned compounds of the invention, namely the glycosylated antibodies described herein.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5587

Said diagnostic composition may comprise the compounds of the invention, in particular the glycosylated antibody molecules of the present invention, soluble form/liquid phase but it is also envisaged that said compounds are bound to/attached to and/or linked to a solid support.

Solid supports may be used in combination with the diagnostic composition as defined herein or the compounds of the present invention may be directly bound to said solid supports. Such supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The compound(s) of the invention, in particular the antibodies of the present invention, may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Appropriate labels and methods for labeling have been identified above and are furthermore mentioned herein below. Suitable methods for fixing/immobilizing said compound(s) of the invention are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like.

It is particularly preferred that the diagnostic composition of the invention is employed for the detection and/or quantification of APP and/or APP-processing products, like amyloid-β or for the detection and/or quantification of pathological and/or (genetically) modified APP-cleavage sides.

As illustrated in the appended examples, the inventive glycosylated antibody molecules are particularly useful as diagnostic reagents in the detection of genuine human amyloid plaques in brain sections of Alzheimer's Disease patients by indirect immunofluorescence.

It is preferred that said compounds of the present invention to be employed in a diagnostic composition are detectably labeled. A variety of techniques are available for labeling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
5588

Commonly used labels comprise, inter alia, fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), enzymes (like horse radish peroxidase, β-galactosidase, alkaline phosphatase), radioactive isotopes (like ^{32}P or ^{125}I), biotin, digoxygenin, colloidal metals, chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums). Labeling procedures, like covalent coupling of enzymes or biotinyl groups, iodinations, phosphorylations, biotinylations, etc. are well known in the art.

Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc. Commonly used detection assays comprise radioisotopic or non-radioisotopic methods. These comprise, inter alia, Westernblotting, overlay-assays, RIA (Radioimmuno Assay) and IRMA (Immune Radioimmunometric Assay), EIA (Enzyme Immuno Assay), ELISA (Enzyme Linked Immuno Sorbent Assay), FIA (Fluorescent Immuno Assay), and CLIA (Chemioluminescent Immune Assay).

Furthermore, the present invention provides for the use of the glycosylated antibody molecules of invention, or an antibody molecule produced by the method of the invention, or a mixture of mono- and double-glycosylation antibodies as provided herein for the preparation of a pharmaceutical or a diagnostic composition for the prevention, treatment and/or diagnosis of a disease associated with amyloidogenesis and/or amyloid-plaque formation. It is further preferred that the compounds described herein, in particular the antibody molecules of the invention, be employed in the prevention and/or treatment of neuropathologies associated with modified or abnormal APP-processing and/or amyloidogenesis. The antibody molecules, e.g. in the format of (engineered) immunoglobulins, like antibodies in a IgG framework, in particular in an IgG1-framework, or in the format of chimeric antibodies (in particular fully humanized antibodies or complete antibodies), bispecific antibodies, single chain Fvs (scFvs) or bispecific scFvs and the like are employed in the preparation of the pharmaceutical compositions provided herein. Yet, the antibody molecules and mixtures provided herein are also useful in diagnostic settings as documented in the appended examples, since the antibody molecules of the invention specifically interact with/detect Aβ4 and/or amyloid deposits/plaques.

Therefore an inventive use of the compounds of the present invention is the use for the preparation of a pharmaceutical composition for a neurological disorder which calls for amelioration, for example by disintegration of β-amyloid plaques, by amyloid (plaque) clearance or by passive immunization against β-amyloid plaque formation. As illustrated in the appended

examples, the inventive antibody molecules are particularly useful in preventing Aβ aggregation and in de-polymerization of already formed amyloid aggregates. Accordingly, the inventive glycosylated antibodies or a mixture of mono- and double-glycosylated antibodies as described herein are to be employed in the reduction of pathological amyloid deposits/plaques, in the clearance of amyloid plaques/plaque precursors as well as in neuronal protection. It is in particular envisaged that the antibody molecules of the invention be employed in the in vivo prevention of amyloid plaques as well as in in vivo clearance of pre-existing amyloid plaques/deposits. Furthermore, the antibody molecules or the mixtures of the invention may be employed in passive immunization approaches against Aβ peptide and aggregates of Aβ, namely amyloid-β plaques. Clearance of Aβ4/Aβ4 deposits may, inter alia, be achieved by the medical use of antibodies of the present invention which comprise an Fc-part. Said Fc-part of an antibody may be particularly useful in Fc-receptor mediated immune responses, e.g. the attraction of macrophages (phagocytic cells and/or microglia) and/or helper cells. For the mediation of Fc-part-related immunoresponses, the antibody molecule of the invention is preferably in an (human) IgG1- framework. As discussed herein, the preferred subject to be treated with the inventive antibody molecules, or antibody mixtures is a human subject. Other frameworks, like IgG2a- or IgG2b-frameworks for the inventive antibody molecules are also envisaged. Immunoglobulin frameworks in IgG2a und IgG2b format are particular envisaged in mouse settings, for example in scientific uses of the inventive antibody molecules, e.g. in tests on transgenic mice expressing (human) wild type or mutated APP, APP-fragments and/or Aβ4.

The above recited diseases associated with amyloidogenesis and/or amyloid-plaque formation comprise, but are not limited to dementia, Alzheimer's disease, motor neuropathy, Parkinson's disease, ALS (amyotrophic lateral sclerosis), scrapie, HIV-related dementia as well as Creutzfeld-Jakob disease, hereditary cerebral hemorrhage, with amyloidosis Dutch type, Down's syndrome and neuronal disorders related to aging. The antibody molecules of the invention and the compositions provided herein may also be useful in the amelioration and or prevention of inflammatory processes relating to amyloidogenesis and/or amyloid plaque formation.

Accordingly, the present invention also provides for a method for treating, preventing and/or delaying neurological and/or neurodegenerative disorders comprising the step of administering to a subject suffering from said neurological and/or neurodegenerative disorder and/or to a subject susceptible to said neurological and/or neurodegenerative disorder an effective amount of a an anti Aβ antibody molecule or a mixture of the inventive mono- and/or double-

glycosylated A-beta antibodies as provided herein and/or a composition as defined herein above. The treatment as provided herein may comprise the administration for the compounds/compositions of this invention alone or in form of a co-therapy treatment, i.e. in combination with other drugs or medicaments. In a particular preferred embodiment of the invention, a method for treating, preventing and/or delaying neurological and/or neurodegenerative disorders is provided that comprises the step of administering to a patient in need of a corresponding medical intervention the antibody mixture comprising mono- and double-glycosylated antibodies directed against Aβ and as provided herein.

The term "treatment" used herein envisages the administration of mono-and/or double-glycosylated antibodies (or mixtures thereof) as described herein to a patient in need thereof. Said patient may be a human patient, in one embodiment an human suffering from or being susceptible to a disorder related to pathological APP processing. Accordingly, the term "treatment" as used herein comprises the prophylactic as well as the curative administration of the compounds or compound mixtures provided herein.

An disorder to be treated by the compounds and composition provided herein is Alzheimer's disease. Patients having a diagnosis of probable Alzheimer's disease based on the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for this diagnosis (NINCDS/ADRDA criteria) Mckhann et al., 1984.

Also envisaged in context of this invention is the medical use of the compounds and/or compositions provided herein in a "co-therapy" setting. for example in the case of APP-related disorders, like Alzheimer's disease. In said case, co-therapy with approved medicaments, like memantine, doneprezil, rivastigmine or galantamine, is envisaged.

In yet another embodiment, the present invention provides for a kit comprising at least one glycosylated antibody molecule as defined herein or the mixture of the inventive mono- and/or double-glycosylated methods as provided herein. Advantageously, the kit of the present invention further comprises, optionally (a) buffer(s), storage solutions and/or remaining reagents or materials required for the conduct of medical, scientific or diagnostic assays and purposes. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The kit of the present invention may be advantageously used, inter alia, for carrying out the method of the invention and could be employed in a variety of applications referred herein, e.g., as diagnostic kits, as research tools or medical tools. Additionally, the kit of the invention may contain means for detection suitable for scientific, medical and/or diagnostic purposes. The manufacture of the kits follows preferably standard procedures which are known to the person skilled in the art.

The figures show:

Figure 1: Plasmid map showing the insertion sites for the heavy and light chain sequences

Figure 2: Example of an analytical chromatogram

Figure 3: Chromatogram of a CMT column as described in the text. Double-glycosylated and mono-glycosylated isoforms are eluting in double peak 1, the non-glycosylated isoform is eluting in peak 2

Figure 4: Whole IgG ESI-MS analysis of ANTIBODY A isoforms. Molecular mass of main peak is indicated in Da. A: non-glycosylated ANTIBODY A; B: mono-glycosylated ANTIBODY A; C: double-glycosylated ANTIBODY A

Figure 5: Scheme of deduced ANTIBODY N-glycosylation patterns. Structures that occur only partially are indicated by parenthesis. A: Complex Type; B: Hybrid Type; C: Oligomannose Type; GlcNAc = N-acetyl-glucosamine, Man = mannose; Gal = galactose; Fuc = fucose; NeuAc = N-acetyl-neuraminic acid

Figure 6: Schematic presentation of carbohydrate structures at Asn306 of ANTIBODY A deduced from MS and HPAEC-PAD analysis. Structures that occur only partially are indicated by parenthesis. GlcNAc = N-acetyl-glucosamine, Man = mannose; Gal = galactose; Fuc = fucose; NeuAc = N-acetyl-neuraminic acid

Figure 7: Binding of ANTIBODY A isoforms to immobilized fibrillar Aβ40 (Biacore sensor chip). Antibody concentration 60 nM. Binding curve of a mixture of all isoforms, i.e. before purification is also shown as indicated.

Figure 8: Epitope mapping of ANTIBODY A COMPOSITION by pepspot analysis. A) pepspot signals of indicated single overlapping decapeptide spots; B) densitometric analysis of signal intensity of single overlapping decapeptide spots.

Figure 9: De-polymerization Assay. ANTIBODY A COMPOSITION and ANTIBODY A isoforms induce release of biotinylated Aβ from aggregated Aβ

Figure 10: ANTIBODY A COMPOSITION and comprising ANTIBODY A isoforms capture soluble Aβ from human cerebrospinal fluid (CSF). Average of 4 CSF samples from Alzheimer's disease patients analyzed in pools of 2. Two immunoprecipitations followed by Western blots per pool with quantification of captured Aβ by densitometry of Western blots. The highest Aβ value on a given series of Western blots was taken as 100%

Figure 11: Indirect immunofluorescence staining of human amyloid plaques with ANTIBODY A isoforms in vitro. Highly sensitive and specific detection of genuine ex vivo human β-amyloid plaques after staining with 10 ng/ml ANTIBODY A concentration. Bound ANTIBODY A was revealed by goat anti-human (H+L)-Cy3 for (A) ANTIBODY A COMPOSITION; (B) double-glycosylated ANTIBODY A; (C) mono-glycosylated ANTIBODY A; and (D) non-glycosylated ANTIBODY A. Scale bar = 80 μm

Figure 12: In vivo immuno-decoration of PS2APP transgenic mouse plaques with glycosylated ANTIBODY A isoforms revealed by confocal microscopy. Immunodecoration reveals in vivo binding of ANTIBODY A isoforms 3 days after a single dose of 1 mg of ANTIBODY A isoforms. Representative images of the distribution of ANTIBODY A isoforms are shown for the double- (A), mono- (B), and non-glycosylated (C) ANTIBODY A isoform. Scale bar = 80 μm

Figure 13: Binding analysis of anti-Aβ antibodies to cell surface APP. Antibody binding to human APP-transfected HEK293 cells and non-transfected control cells analyzed by flow cytometry.

Figure 14: Scheme of ANTIBODY A non-, mono- and double-glycosylated antibody molecules (immunoglobulins).

Figure 15: Total plaque surface (A), total plaque number (B) and plaque number and size distribution (C) in the thalamus region after 5 month treatment with ANTIBODY A COMPOSITION (which comprises mono- and double-glycosylated ANTIBODY A), double-glycosylated and mono-glycosylated ANTIBODY A isoforms (20 mg/kg weekly, i.v.) or vehicle.

Figure 16: Total plaque surface (A), total plaque number (B) and plaque number and size distribution (C) in the cortex and corpus callosum region after 5 month treatment with ANTIBODY A COMPOSITION (which comprises mono- and double-glycosylated ANTIBODY A), double-glycosylated and mono-glycosylated ANTIBODY A isoforms (20 mg/kg weekly, i.v.) or vehicle.

Figure 17: Total plaque surface (A), total plaque number (B) and plaque number and size distribution (C) in the hippocampus region after 5 month treatment with ANTIBODY A COMPOSITION (which comprises mono- and double-glycosylated ANTIBODY A), double-glycosylated and mono-glycosylated ANTIBODY A isoforms (20 mg/kg weekly, i.v.) or vehicle.

Figure 18: Total plaque surface (A), total plaque number (B) and plaque number and size distribution (C) in the subiculum region after 5 month treatment with ANTIBODY A COMPOSITION (which comprises mono- and double-glycosylated ANTIBODY A), double-glycosylated and mono-glycosylated ANTIBODY A isoforms (20 mg/kg weekly, i.v.) or vehicle.

Figure 19: Measurement of fluorescence intensity of immunostained ANTIBODY A COMPOSITION bound to amyloid-β plaques after biweekly dosing of 0.1 mg/kg with 1, 2 and 4 i.v. applications to PS2APP mice. Analysis was done at 2 weeks after last injection.

Figure 20: Measurement of fluorescence intensity of immunostained ANTIBODY A COMPOSITION bound to amyloid-β plaques after monthly dosing of 0.15 mg/kg with 2 and 3 i.v. applications to PS2APP mice. Analysis was done at 2 weeks after last injection.

Figure 21: Measurement of fluorescence intensity of immunostained ANTIBODY A COMPOSITION bound to amyloid-β plaques after 4 biweekly injections of 0.05, 0.1 and 0.30 mg/kg to PS2APP mice, suggesting dose-related amyloid-plaque binding. Analysis was done at 2 weeks after last injection.

Figure 22: Measurement of fluorescence intensity of immunostained ANTIBODY A COMPOSITION bound to amyloid-β plaques after 3 monthly injections of 0.075, 0.15 and 0.45 mg/kg to PS2APP mice, suggesting dose-related amyloid-plaque binding. Analysis was done at 2 weeks after last injection.

Figure 23: Human AD brain sections stained against Aβ with anti-Aβ murine monoclonal antibody (BAP-2) after 40 hours incubation with ANTIBODY A COMPOSITION at indicated concentrations together with living differentiated primary human macrophages (0.8 million cells/ml). Results show reduction in amyloid load indicative for antigen-dependent cellular phagocytosis effect of ANTIBODY A COMPOSITION on amyloid-β plaques. Scale bar = 300 μm.

Figure 24: Dose response of ANTIBODY A COMPOSITION on amyloid-β plaques from human AD brain sections when incubated with 0.8 million cells/ml. (A) shows total plaque area and (B) staining intensity.

Figure 25: Fluorescent microscopy of P388D1 cells incubated with 0, 0.1, 1 and 10 μg/ml ANTIBODY A COMPOSITION (A to D, respectively).

Figure 26: Quantitative measurement of dose response of ANTIBODY A COMPOSITION using Aβ conjugated fluorobeads and P3881D1 cells (shown in relative fluorescent units, RFU). Two independent experiments indicate a considerable range of efficacy for ANTIBODY A COMPOSITION.

Figure 27: Table showing different glycan structures of ANTIBODY A in the constant region of the heavy chain (Asn 306; first two columns) and in the variable region of the heavy chain (Asn 52; third and fourth column).

Examples

The following, non-limiting examples illustrate the invention.

Example 1: Generation of ANTIBODY A via cloning techniques

In accordance with the present invention, an IgG1 molecule was generated via common cloning techniques. ANTIBODY A is, in its coding sequence and in its expressed amino acid sequence characterized by its variable region of the heavy chain (V_H). The corresponding example of a heavy encoded by a DNA sequence as follows:

caggtggaattggtggaaagcggcggcggcctggtgcaaccgggcggcagcctgcgtctgagctgcgcggcctccggatttacctttag
cagctatgcgatgagctgggtgcgccaagcccctgggaagggtctcgagtgggtgagcgctattaatgcttctggtactcgtacttattatgc
tgattctgttaagggtcgttttaccatttcacgtgataattcgaaaaacaccctgtatctgcaaatgaacagcctgcgtgcggaagatacggcc
gtgtattattgcgcgcgtggtaagggtaatactcataagccttatggttatgttcgttattttgatgtttggggccaaggcaccctggtgacggtt
agctcagcctccaccaagggtccatcggtcttcccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacag
tcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc
ccagcaacaccaaggtggacaagaaagttgagcccagatatcgtgcgatatcgtgcaatcttgtgacaaaactcacacatgcccaccgtgc
ccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagaca
aagccgcgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagt
acaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
gtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctaca
gcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaatga (SEQ ID NO: 5).

and codes for the following immunoglobulin H-chain:

QVELVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINASGTRT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVRYFDV
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 6)

The same heavy chain may also be encoded by a sequence comprising an additional "leader sequence" as shown in the following sequence

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtcctgtcc
caggtggaattggtggaaagcggcggcggcctggtgcaaccgggcggcagcctgcgtctgagctgcgcggcctccggatttacctttag
cagctatgcgatgagctgggtgcgccaagcccctgggaagggtctcgagtgggtgagcgctattaatgcttctggtactcgtacttattatgc
tgattctgttaagggtcgttttaccatttcacgtgataattcgaaaaacaccctgtatctgcaaatgaacagcctgcgtgcggaagatacggcc
gtgtattattgcgcgcgtggtaagggtaatactcataagccttatggttatgttcgttattttgatgtttggggccaaggcaccctggtgacggtt
agctcagcctccaccaagggtccatcggtcttcccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacag
tcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc
ccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgg
ggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgt
gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatc
ccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagca
atgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggaca
agagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgt
ctccgggtaaatga (SEQ ID NO: 25)

The corresponding amino acid sequence would be

MKHLWFFLLLVAAPRWVLS
QVELVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINASGTRT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVRYFDV
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 26)

Similarly, the light chain of ANTIBODY A is encoded by the following nucleotide sequence:

gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcgagccagagcgtga
gcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatggcgcgagcagccgtgcaactggggtcc
cggcgcgttttagcggctctggatccggcacggattttaccctgaccattagcagcctggaacctgaagactttgcgacttattattgccttca
gatttataatatgcctattacctttggccagggtacgaaagttgaaattaaacgtacggtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg
ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggg
gagagtgttag (SEQ ID NO: 7)

and codes for the following amino acid sequence (L-chain):

DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVP
ARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 8)

Again, also here, a "leader sequence" may be employed and the corresponding sequences would be

atggtgttgcagacccaggtcttcatttctctgttgctctggatctctggtgcctacggg
gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcgagccagagcgtga
gcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatggcgcgagcagccgtgcaactggggtcc
cggcgcgttttagcggctctggatccggcacggattttaccctgaccattagcagcctggaacctgaagactttgcgacttattattgccttca
gatttataatatgcctattacctttggccagggtacgaaagttgaaattaaacgtacggtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg
ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggg
gagagtgttag (SEQ ID NO: 27)

This sequence encodes the following amino acid sequence

MVLQTQVFISLLLWISGAYG
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVP
ARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRTVAAPSVFIFPPS

DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 28)

These sequences above are known from MAB31 as disclosed in WO 03/070760.

However, the heavy and light chains of the exemplified ANTIBODY A may also be encoded by a sequence as shown below:

a) the heavy chain

atggagtttgggctgagctgggttttcctcgttgctcttttaagaggtgattcatggagaaatagagagactgagtgtgagtg
aacatgagtgagaaaaactggatttgtgtggcattttctgataacggtgtccttctgtttgcaggtgtccagtgtcaggtggag
ctggtggagtctgggggaggcctggtccagcctgggggtccctgagactctcctgtgcagcgtctggattcaccttcagt
agctatgccatgagctgggtccgccaggctccaggcaaggggctcgagtgggtgtccgccataaacgccagcggtacc
cgcacctactatgcagactccgtgaagggccgattcaccatctccagagacaattccaagaacacgctgtatctgcaaatg
aacagcctgagagccgaggacacggctgtgtattactgtgcgagaggcaaggggaacacccacaagccctacggctac
gtacgctactttgacgtgtggggccaaggaaccctggtcaccgtctcctcaggtgagtcctcacaacctctcctcgcggc
cgcagcttgaagtctgaggcagaatcttgtccagggtctatcggactcttgtgagaattaggggctgacagttgatggtgac
aatttcagggtcagtgactgtctggtttctctgaggtgagactggaatataggtcaccttgaagactaaagaggggtccagg
ggcttttctgcacaggcagggaacagaatgtggaacaatgacttgaatggttgattcttgtgtgacaccaagaattggcata
atgtctgagttgcccaagggtgatcttagctagactctggggtttttgtcgggtacagaggaaaaacccactattgtgattact
atgctatggactactggggtcaaggaacctcagtcaccgtctcctcaggtaagaatggcctctccaggtctttatttttaacct
ttgttatggagttttctgagcattgcagactaatcttggatatttgccctgagggagccggctgagagaagttgggaaataaa
tctgtctagggatctcagagcctttaggacagattatctccacatctttgaaaaactaagaatctgtgtgatggtgttggtgga
gtccctggatgatgggatagggactttggaggctcatttgagggagatgctaaaacaatcctatggctggagggatagttg
gggctgtagttggagattttcagttttagaatgaagtattagctgcaatacttcaaggaccacctctgtgacaaccatttata
cagtatccaggcatagggacaaaaagtggagtggggcactttctttagatttgtgaggaatgttccacactagattgtttaaa
acttcatttgttggaaggagctgtcttagtgattgagtcaagggagaaaggcatctagcctcggtctcaaaagggtagttgct
gtctagagaggtctggtggagcctgcaaaagtccagctttcaaaggaacacagaagtatgtgtatggaatattagaagatg
ttgcttttactcttaagttggttcctaggaaaaatagttaaatactgtgactttaaaatgtgagagggttttcaagtactcatttttt
aaatgtccaaaatttttgtcaatcaatttgaggtcttgtttgtgtagaactgacattacttaaagtttaaccgaggaatgggagtg
aggctctctcataccctattcagaactgacttttaacaataataaattaagtttaaaatatttttaaatgaattgagcaatgttgagt
tgagtcaagatggccgatcagaaccggaacacctgcagcagctggcaggaagcaggtcatgtggcaaggctatttggg
gaagggaaaataaaaccactaggtaaacttgtagctgtggtttgaagaagtggttttgaaacactctgtccagccccaccaa
accgaaagtccaggctgagcaaaacaccacctgggtaatttgcatttctaaaataagttgaggattcagccgaaactggag

aggtcctcttttaacttattgagttcaaccttttaattttagcttgagtagttctagtttccccaaacttaagtttatcgacttctaaaa
tgtatttagaattcgagctcggtacagctttctggggcaggccaggcctgaccttggctttggggcagggaggggctaa
ggtgaggcaggtggcgccagcaggtgcacacccaatgcccatgagcccagacactggacgctgaacctcgcggacag
ttaagaacccaggggcctctgcgcctgggcccagctctgtcccacaccgcggtcacatggcaccacctctcttgcagcct
ccaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcct
ggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg
gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctac
atctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttggtgagaggccagcacagggagggag
ggtgtctgctggaagccaggctcagcgctcctgcctggacgcatcccggctatgcagccccagtccagggcagcaagg
caggccccgtctgcctcttcacccggagcctctgcccgccccactcatgctcagggagagggtcttctggctttttcccag
gctctgggcaggcacaggctaggtgcccctaacccaggccctgcacacaaaggggcaggtgctgggctcagacctgc
caagagccatatccgggaggaccctgcccctgacctaagcccaccccaaaggccaaactctccactccctcagctcgga
caccttctctcctcccagattccagtaactcccaatcttctctctgcagagcccaaatcttgtgacaaaactcacacatgccca
ccgtgcccaggtaagccagcccaggcctcgccctccagctcaaggcgggacaggtgccctagagtagcctgcatccag
ggacaggccccagccgggtgctgacacgtccacctccatctcttcctcagcacctgaactcctgggggggaccgtcagtct
tcctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagc
cacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggag
gagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtaca
agtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggtgggacccgtgggg
tgcgagggccacatggacagaggccggctcggcccaccctctgccctgagagtgaccgctgtaccaacctctgtcccta
cagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctg
acctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac
aagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtcccc
gggcaaatga (SEQ ID NO: 23)

b) the light chain

atggacatgagggtcctcgctcagctcctggggctcctgctgctctgtttcccaggtaaggatggagaacactagcagttta
ctcagcccagggtgctcagtactgctttactattcagggaaattctcttacaacatgattaattgtgtggacatttgtttttatgttt
ccaatctcaggcgccagatgtgatatcgtgttgacgcagtctccagccaccctgtctttgtctccaggggaaagagccacc
ctctcctgccgggccagtcagagtgttagcagcagctacttagcctggtaccagcagaaacctggccaggcgcccaggc
tcctcatctatggcgcatccagcagggccactggcgtgccagccaggttcagtggcagtgggtctgggacagacttcact
ctcaccatcagcagcctggagcctgaagatttcgcgacctattactgtctgcagatttacaacatgcctatcacgttcggcca