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Winston, Jr. et al.

(54) HEPATOCYTE GROWTH FACTOR (HGF) BINDING ANTIBODY

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- (51) Int. Cl. *C07K 16/00* (2006.01) *C07K 16/24* (2006.01)
- *C07K 16/24* (2006.01) (52) U.S. Cl. 530/388.24; 530/387.1;
- 530/388.1; 530/388.15 (58) Field of Classification Search None
- See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides a family of binding proteins that bind and neutralize the activity of hepatocyte growth factor (HGF), in particular human HGF. The binding proteins can be used as diagnostic and/or therapeutic agents. With regard to their therapeutic activity, the binding proteins can be used to treat certain HGF responsive disorders, for example, certain HGF responsive tumors.

9 Claims, 9 Drawing Sheets



CDR1 CDR2	GGSLKLSCAASEFTFSNYYMSJWVRQTPEKRLQWVAYISPGGGSSYYPASVKGRFTISRDNAKNTLYL GTSVKLSCKASGYTFTTYYLHWVNQRPGQGLEWIGEINPTNGHTNYNEKFKSKATLTVDKSSSTAYM GTSVKMSCKASGYTFTTYYLHWVNQRPGQGLEWIGEINPTNGHTNYNEKFKSKATLTVDTSSSTAYM SSSVKISCKASGYVFSSYMMNWVKQRPGQGLEWIGEINGKIGPGSSTYYNEMFKDKATLTVDTSSSTAYM SQSLSITCTVSGFSLTSSYMMWVKQRPGQGLEWIGUIWAG-GNTNYNSSLMSKLTIRKDNSKSQVFL GGSLKLSCAASGFTFSDYYMSJWVRQTPEKRLEWVAYISSGGGSTYYPDSVKGRFTISRDNAKNTLYL GGSLKLSCAASGFTFSDYYMSJWVRQTPEKRLEWVAYISSGGGSTYYPDSVKGRFTISRDNAKNTLYL GGSLKLSCAASGFTFSNYFMSJWVRQTPEKRLEWVAYISSGGGSTYYPDSVKGRFTISRDNAKNTLYL GGSLKLSCAASGFTFSNYFMSJWVRQTPEKRLEWVAYISSGGGSTYYPDSVKGRFTISRDNAKNTLYL GGSLKLSCAASGFTFSNYFMSJWVRQTPEKRLEWVAYISSGGGSTYYPDSVKGRFTISRDNAKNTLYL		AMDYWGQGTTLTVSS (SEQ ID NO: 2) IFDYWGQGTTLTVSS (SEQ ID NO: 12) GFDYWGQGTTLTVSS (SEQ ID NO: 22) YFDYWGQGTTLTVSS (SEQ ID NO: 32) -FAYWGQGTLVTVSA (SEQ ID NO: 42) AMDYWGQGTSVTVSS (SEQ ID NO: 42) AMDYWGQGTSVTVSS (SEQ ID NO: 52) AMDYWGQGTSVTVSS (SEQ ID NO: 52)
Signal Peptide	NFGLRLIFLVLVLKGVKCEVQLVESGGGLVQP GWSYIILFLVATATDVHSQVQLQQPGAELVKPC EWSWVFLFLSVTAGVHCQVQLKQSGAELVKPC EWPCIFLFLSVTEGVHSQVQLQQSGAELVRPC AVPVLFLCLVAFPSCVLSQVQLKESGPGLVAPS NFGLRLIFLVLVLKGVKCEVQLVESGGGLVQPC NFGLRLIFLVLVLKGVKCEVQLVESGGGLVQSG NFGLRLIFLVLVLKGVKCEVQLVESGGGLVQSC	CDR3	nt.) QMSSLKSEDTAMYYCARQGDGYYGDY nt.) QLSSLTSEDSAVYYCARNYVGS1 nt.) QLSSLTSEDSAVYFCARKGLGRC nt.) QLSSLTSEDSAVYFCARGGLGRC nt.) QLSSLTSEDSAVYFCARGGGRC nt.) QLSSLTSEDTAMYYCARGF nt.) QMSSLKSEDTAMYYCVRQGDGYYGDYA nt.) QMSSLKSEDTAMYYCVRQGDGYYGDYA nt.) QMSSLKSEDTAMYYCVRQGDGYYGDYA
Antibody	1 1 2 2 3 2 1 1 1 1 2 2 2 2 2 2 2 2 2 2		(1A3 C0 (2B8 C0 (2F8 C0 (3B6 C0 (3D11 C0 (3D11 C0 (1D3 C0 (1F3 C0 (3A12 C0)

Complete Heavy Chain Variable Region Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3	
1A3	NYYMS (SEQ ID NO: 5)	VISPGGGSSYYPASVKG (SEQ ID NO: 6)	QGDGYYGDYAMDY (SEQ ID NO:
2B8	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFKS (SEQ ID NO: 16)	NY~~~VGSIFDY (SEQ ID NO:
2F8	TYYIH (SEQ ID NO: 25)	KIGPGSGSTYYNEMFKD (SEQ ID NO: 26)	RG~~~LGRGFDY (SEQ ID NO:
3B6	SYWMN (SEQ ID NO: 35)	QIYPGDGDSNYNGNFKG (SEQ ID NO: 36)	QLG~~LRENYFDY (SEQ ID NO:
3D11	SYSLH (SEQ ID NO: 45)	VIWAG~GNTNYNSSLMS (SEQ ID NO: 46)	ER~~~~~FAY (SEQ ID NO:
1D3	DYYMS (SEQ ID NO: 55)	VISSGGGSTYYPDSVKG (SEQ ID NO: 56)	OGDGYYGDYAMDY	SEQ ID NO:
1F3	NYFMS (SEQ ID NO: 65)	VISSGGGSTYYPDSVKG (SEQ ID NO: 66)	QGDGYYGDYAMDY	SEQ ID NO:
3A12	NYFMS (SEQ ID NO: 75)	VISSGGGSTYYPDSVKG (SEQ ID NO: 76)	OGDGYYGDYAMDY (SEQ ID NO:

Heavy Chain CDR Amino Acid Alignments

FIG. 3

7) 17) 27) 37) 57) 67)

on Amino Acid Alignments	CDR2	NILAWYQQKQGKSPQLLVYAATNILADGVPSRFSGSGSGTQFSLK iYVSWYQQKPAQSPKLLIYGASNRNTGVPDRFTGSGSGTDFTLN YLINWYQQKPGQPPKVLIYVASNLESGIPARFSGSGSGGSGTDFTLN iYLSWFQQKPGKSPKTLIYRVNRLVDGVPSRFSGSGSGGSGTSYSLT iNLAWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGGSGTOFSLR INLAWYQQKSGTSPKRWIYDTSKLASGVPSRFSGSGSGTQFSLR INLAWYQQKQGKSPQLLVYDATHLPDGVPSRFSGSGSGTQFSLK INLAWYQQKQGKSPQLLVHAATKLADGVPSRFSGSGSGTQFSLK		
ete Light (Kappa) Chain Variable Regic	de CDR1	WLTDARCDIQMTQSPASLSVSVGETVTITCRASENIYS WLYGADGNIVMTQSPKSMSMSVGERVTLSCRASENVVS WVPGSTGDIVLTQSPASLAVSLGQRATISCRASQSVDYDGNS WFPGIKCDIKMTQSPSSMYASLGERVTITCRASQSVDYDGNS WFPGIKCDIKMTQSPSSMYASLGERVTITCRASQDIKS WLTDVRCDIQMTQSPASLSVSVGETVTITCRASENIYS WLTDARCDIQMTQSPASLSVSVGETVTITCRASENIYII WLTDARCDIQMTQSPASLSVSVGETVTITCRASENIYII	CDR3	FGTYYCQHFWGTPYTFGGGTKLEIK (SEQ ID NO: 4) LADYHCGQSYNYPYTFGGGTRLEIK (SEQ ID NO: 14) AATYYCQQSIEDPPTFGGGTKLEIK (SEQ ID NO: 24) MGIYYCLQYDEFPFTFGGGTKLEIK (SEQ ID NO: 24) AATYYCQQWSSNPLTFGGGTKLEIK (SEQ ID NO: 44) FGRYYCQHFWGTPYTFGGGTKLEIK (SEQ ID NO: 54) FGSYYCQHFWGTPYTFGGGTKLEIK (SEQ ID NO: 54) FGSYYCQHFWGTPYTFGGGTKLEIK (SEQ ID NO: 54)
Comple	ly Signal Peptid	MSVPTQVLGLLLLM - MESQTLVFISILLM - MDMRTPAQFLGILLLM MDPQVQIFSFLLISAS - MSVPTQVLGLLLLM - MSVPTQVLGLLLLM - MSVPTQVLGLLLLM		cont.) INSLQSEDF cont.) ISSVRAEDI cont.) IHPVEEEDA cont.) ITSLENEDM cont.) INSLQSEDF cont.) INSLQSEDF cont.) INSLQSEDF cont.) INSLQSEDF
	Antiboc	1A3 2B8 2F8 3B6 3D11 1D3 1F3 3A12 3A12		(1A3 (2B8 (2F8 (3D11 (1D3 (1F3 (1F3 (3A12

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FIG. 4

Antibody	CDR1	CDR2	CDR3
1A3	RASENIY ~~~~SNLA (SEQ ID NO: 8)	AATNLAD (SEQ ID NO: 9)	QHFWGTPYT (SEQ ID NO: 10)
1D3	RTSENIY~~~~SNLA (SEQ ID NO: 58)	AATNLAD (SEQ ID NO: 59)	QHFWGTPYT (SEQ ID NO: 60)
2B8	KASENVV~~~~SYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)
2F8	KASQSVDYDGNSYIN (SEQ ID NO: 28)	VASNLES (SEQ ID NO: 29)	QQSIEDPPT (SEQ ID NO: 30)
3D11	SASSSVS~~~~YMH (SEQ ID NO: 48)	DTSKLAS (SEQ ID NO: 49)	QQWSSNPLT (SEQ ID NO: 50)
3B6	KASQDIK~~~~SYLS (SEQ ID NO: 38)	RVNRLVD (SEQ ID NO: 39)	LQYDEFPFT (SEQ ID NO: 40)
1F3	RASENIY~~~~SNLA (SEQ ID NO: 68)	DATHLPD (SEQ ID NO: 69)	QHFWGTPYT (SEQ ID NO: 70)
3A12	RASENIY~~~~INLA (SEQ ID NO: 78)	AATKLAD (SEQ ID NO: 79)	QHFWGTPYT (SEQ ID NO: 80)

FIG. 5

Light (Kappa) Chain CDR Amino Acid Alignments





Kappa	Heavy	ka (1/Ms)	STDEV	kd (1/s)	STDEV	KD (pM)	STDEV
Chimeric 2B8	Chimeric 2B8	2.3x10 ⁶		2.7×10 ⁻⁵		11.6	
Hu2B8_Kv1-39.1	Chimeric 2B8	2.8x10 ⁶		3.9x10 ⁻⁵		13.6	
Hu2B8_Kv3-15.1	Chimeric 2B8	3.1x10 ⁶		1.7x10 ⁻⁵		5.5	
Chimeric 2B8	Hu2B8_Hv1-f.1	2.4x10 ⁶		1.6x10 ⁻³		662.5	
Chimeric 2B8	Hu2B8_Hv5-a.1	2.4x10 ⁶		1.1x10 ⁻⁵		4.4	
Chimeric 2B8	Hu2B8_Hv5-51.1	2.1x10 ⁶		3.4x10 ⁻⁵		16.3	
Hu2B8_Kv1-39.1	Hu2B8_Hv1-f.1	7.1x10 ⁶		2.1x10 ⁻³		294.0	
Hu2B8_Kv1-39.1	Hu2B8_Hv5-a.1	2.6x10 ⁶		3.8x10 ⁻⁵		14.7	
Hu2B8_Kv1-39.1	Hu2B8_Hv5-51.1	2.0x10 ⁶	4.2x10 ⁵	1.7x10 ⁻⁵	1.4x10 ⁻⁵	8.1	5.3
Hu2B8_Kv3-15.1	Hu2B8_Hv1-f.1	7.8x10 ⁶		3.7x10 ⁻³		465.8	
Hu2B8_Kv3-15.1	Hu2B8_Hv5-a.1	2.2x10 ⁶		5.9x10 ⁻⁵		26.9	
Hu2B8_Kv3-15.1	Hu2B8_Hv5-51.1	1.9x10 ⁶	4.7x10 ⁵	2.3x10 ⁻⁵	6.3x10 ⁻⁶	12.0	0.4

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Fig. 8



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Fig. 9

HEPATOCYTE GROWTH FACTOR (HGF) BINDING ANTIBODY

RELATED APPLICATIONS

This application claims the benefit and priority to U.S. Provisional Application Nos. 60/810,714, filed Jun. 2, 2006, and 60/860,509, filed Nov. 21, 2006, the disclosures of which are incorporated by reference herein.

FIELD OF THE INVENTION

The field of the invention is molecular biology, immunology and oncology. More particularly, the field is antibody-15 based binding proteins that bind human hepatocyte growth factor (HGF).

BACKGROUND

20 Hepatocyte Growth Factor (HGF), also known as Scatter Factor (SF), is a multi-functional heterodimeric protein produced predominantly by mesenchymal cells, and is an effector of cells expressing the Met tyrosine kinase receptor (Bottaro et al. (1991) SCIENCE 251: 802-804, Rubin et al. (1993) 25 BIOCHIM. BIOPHYS. ACTA 1155: 357-371). The human Met receptor is also known as "c-Met." Mature HGF contains two polypeptide chains, the α -chain and the β -chain. Published studies suggest it is the α -chain that contains HGF's c-Met receptor binding domain.

When it binds to its cognate receptor, HGF mediates a number of cellular activities. The HGF-Met signaling pathway plays a role in liver regeneration, wound healing, neural regeneration, angiogenesis and malignancies. See, e.g., Cao et al. (2001) PROC. NATL. ACAD. SCI. USA 98: 7443-7448, 35 Burgess et al. (2006) CANCER RES. 66: 1721-1729, and U.S. Pat. Nos. 5,997,868 and 5,707,624. Investigators have been developing a number of HGF modulators, including antibodies, to treat various disorders that involve HGF activity, for example, certain HGF responsive cancers. See, e.g., International Application Publication No. WO 2005/017107.

The basic structure common to all antibodies is shown schematically in FIG. 1. Antibodies are multimeric proteins that contain four polypeptide chains. Two of the polypeptide chains are called heavy or H chains and two of the polypeptide $_{45}$ chains are called light or L chains. The immunoglobulin heavy and light chains are connected by an interchain disulfide bond. The immunoglobulin heavy chains are connected by a number of interchain disulfide bonds. A light chain is composed of one variable region (V $_L$ in FIG. 1) and one $_{50}$ constant region (C_L in FIG. 1), while the heavy chain is composed of one variable region (V_H in FIG. 1) and at least three constant regions (CH₁, CH₂ and CH₃ in FIG. 1). The variable regions determine the specificity of the antibody and the constant regions have other functions.

Amino acid and structural information indicate that each variable region comprises three hypervariable regions (also known as complementarity determining regions or CDRs) flanked by four relatively conserved framework regions or FRs. The three CDRs, referred to as CDR_1 , CDR_2 , and CDR_3 , 60 are responsible for the binding specificity of individual antibodies. When antibodies are to be used as diagnostic and therapeutic agents, typically it is desirable to create antibodies that have the highest binding specificity and affinity to the target molecule. It is believed that differences in the variable regions can have profound effects on the specificity and affinity of the antibody.

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U.S. Pat. No. 5,707,624 describes the use of anti-HGF antibodies in the treatment of Kaposi's sarcoma. Similarly, U.S. Pat. No. 5,997,868 describes treating a tumor by administering an anti-HGF antibody to the patient to be treated so as to block the ability of endogeneous HGF to promote angiogenesis in the tumor. More recently, investigators propose that antibodies that bind the β -chain of HGF may have potential as therapeutic agents in patients with HGF-dependent tumors (Burgess (2006) supra).

Notwithstanding, there is still a need for additional HGF 10modulators that can be used as therapeutic and diagnostic agents.

SUMMARY OF THE INVENTION

The invention is based, in part, upon the discovery of a family of binding proteins that specifically bind HGF, in particular, human HGF. The binding proteins are antibodybased in so far as they contain antigen (i.e., HGF) binding sites based on the CDRs of a family of antibodies that specifically bind HGF. The CDRs confer the binding specificity of the binding proteins to HGF. The binding proteins can be used as diagnostic and therapeutic agents. When used as a therapeutic agent, the binding proteins are engineered (e.g., humanized) so as to reduce or eliminate the risk of inducing an immune response against the binding protein when administered to the recipient (e.g., a human).

The binding proteins neutralize the activity of HGF and, therefore, can be used as a therapeutic agent. In certain embodiments, the binding proteins prevent HGF from binding to its cognate receptor, c-Met, thereby neutralizing HGF activity. In other embodiments, the binding proteins bind to HGF and neutralize its biological activity but without preventing HGF from binding to the c-Met receptor. Because HGF has been implicated in the growth and proliferation of cancer cells, the binding proteins can be used to inhibit the proliferation of cancer cells. Furthermore, when administered to a mammal, the binding proteins can inhibit or reduce tumor growth in the mammal.

These and other aspects and advantages of the invention will become apparent upon consideration of the following figures, detailed description, and claims.

DESCRIPTION OF THE DRAWINGS

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

FIG. 1 is a schematic representation of a typical antibody. FIG. 2 is a schematic diagram showing the amino acid sequence defining the complete immunoglobulin heavy chain variable region of the antibodies denoted as 1A3, 1 D3, 1F3, 2B8, 2F8, 3A12, 3B6 and 3D11. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR₁, CDR₂, and CDR₃ 55 are identified in boxes. The unboxed sequences represent FR sequences.

FIG. 3 is a schematic diagram showing the CDR_1 , CDR_2 , and CDR₃ sequences for each of the immunoglobulin heavy chain variable region sequences presented in FIG. 2.

FIG. 4 is a schematic diagram showing the amino acid sequence defining the complete immunoglobulin light chain variable region of the antibodies 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6, and 3D11. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR₁, CDR₂, and CDR₃ are identified in boxes. The unboxed sequences represent FR sequences.

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FIG. 5 is a schematic diagram showing the CDR_1 , CDR_2 , and CDR₃ sequences for each of the immunoglobulin light chain variable region sequences presented in FIG. 4.

FIG. 6 is a graph summarizing results from an experiment to measure tumor inhibitory activity of anti-HGF antibodies 1D3, 1F3, 1A3 and 2B8 in a U87MG xenograft model. Diamonds correspond to PBS; triangles correspond to anti-HGF antibody 1A3; X corresponds to anti-HGF antibody 1D3; squares correspond to anti-HGF antibody 1F3, and circles 10 correspond to anti-HGF antibody 2B8.

FIG. 7 is a graph summarizing results from an experiment to measure tumor inhibitory activity of anti-HGF antibodies 1D3, 1F3, 1A3 and 2B8 in a U118 xenograft model. Diamonds correspond to IgG; squares correspond to anti-HGF antibody 1F3, triangles to anti-HGF antibody 1D3; X corresponds to anti-HGF antibody 1A3; and circles correspond to anti-HGF antibody 2B8.

FIG. 8 is a table summarizing surface plasmon resonance data on antigen-binding affinity and kinetics of interaction between human HGF and chimeric, chimeric/humanized, or humanized 2B8 antibodies. The table lists the pairs of Kappa light chain and IgG1 heavy chain tested. Those antibodies with standard deviations (STDEV) listed were analyzed in 25 three independent experiments.

FIG. 9 is a bar chart summarizing experimental data indicating that Hu2B8 binds an epitope mutually exclusive to murine monoclonal antibody 2B8. Humanized or chimeric 2B8 was captured on an anti-human Fc chip. HGF then was 30 bound to the humanized or chimeric 2B8. The ability of mouse 2B8 or the control antibody (polyclonal goat anti-HGF antibody) to bind the captured HGF was measured. Both humanized 2B8 antibodies and chimeric 2B8 prevent murine 2B8 from binding HGF. White bars correspond to the chimeric 2B8 antibody; gray bars correspond to the humanized Hu2B8 antibody (kappa variable region Kv1-39.1 and heavy chain variable region Hv5-51.1); black bars correspond to the humanized Hu2B8 antibody (kappa variable region Kv3-15.1 and heavy chain variable region Hv5-51.1).

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of a 45 family of binding proteins that specifically bind, and neutralize the activity of, HGF, in particular, human HGF. The binding proteins can be used in a variety of diagnostic and therapeutic applications. The binding proteins are based upon the antigen binding sites of certain monoclonal antibodies that 50 have been selected for their ability to bind, and neutralize the activity of, HGF. In particular, the binding proteins contain immunoglobulin variable region CDR sequences that together define a binding site for HGF.

In view of the neutralizing activity of these antibodies, they 55 are particularly useful in modulating the growth and/or proliferation of HGF responsive cells, for example, cancer cells. When used as a therapeutic agent, the binding proteins can be engineered so as to minimize or eliminate the risk of inducing an immune response against the binding proteins when 60 administered to the recipient. Furthermore, depending upon the particular application, it is contemplated that the binding proteins can be conjugated to other moieties, for example, detectable labels, for example, radiolabels, and effector molecules, for example, other protein and small molecule-based 65 therapeutics. Each of these features and aspects of the invention are discussed in more detail below.

I-Binding Proteins that Bind HGF

In one aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region comprising the structure CDR_{L1} - CDR_{L2} - CDR_{L3} , and (ii) an immunoglobulin heavy chain variable region comprising three complementarity determining regions (CDRs), wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF. CDR_{L1} comprises the amino acid sequence X₁ X₂ Ser X₄ X₅ X₆ X₇ X₈ X₉ $X_{10}\,X_{11}\,X_{12}\,X_{13}\,X_{14}\,X_{15},$ wherein amino acid X_1 is Arg, Lys, or Ser, X₂ is Ala or Thr, X₄ is Glu, Gln, or Ser, X₅ is Asn, Asp, or Ser, X₆ is Ile or Val, X₇ is Asp, Lys, Ser, Val, or Tyr, X₈ is a peptide bond or Tyr, X_9 is a peptide bond or Asp, X_{10} is a peptide bond or Gly, X11 is a peptide bond or Asn, X12 is a peptide bond, Ile, or Ser, X13 is Asn or Tyr, X14 is Ile, Leu, Met, or Val, X15 is Ala, Asn, His, or Ser. CDRL2 comprises the amino acid sequence X16 X17 X18 X19 X20 X21 X22, wherein amino acid X₁₆ is Ala, Asp, Arg, Gly, or Val, X₁₇ is Ala, Thr, or Val, X₁₈ is Asn, Ser, or Thr, X₁₉ is Arg, Asn, Lys, or His, X₂₀ is Leu or Arg, X21 is Ala, Asn, Glu, Val, or Pro, X22 is Asp, Ser, or Thr. CDR_{L3} comprises the amino acid sequence $X_{23} X_{24}$ $X_{25} X_{26} X_{27} X_{28}$ Pro X_{30} Thr, wherein amino acid X_{23} is Leu, Gly, or Gln, X_{24} is His or Gln, X_{25} is Phe, Ser, Trp, or Tyr, X_{26} is Asp, Ile, Ser, Trp, or Tyr, $\rm X_{27}$ is Gly, Glu, Asn, or Ser, $\rm X_{28}$ is Asp, Asn, Phe, Thr, or Tyr, X₃₀ is Leu, Phe, Pro, or Tyr.

In another aspect, the invention provides an isolated binding protein that binds human HGF comprising (i) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1} - CDR_{H2} - CDR_{H3} and (ii) an immunoglobulin light chain variable region comprising three complementarity determining regions (CDRs), wherein the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region together define a single binding site for binding human HGF. CDR_{H1} comprises the amino acid sequence X1 Tyr X3 X4 X5, wherein amino acid X1 is Asp, Asn, Ser, or Thr, X₃ is Phe, Ser, Trp, or Tyr, X₄ is Ile, Leu, or Met, X₅ is Asn, His, or Ser. CDR_{H2} comprises the amino acid sequence ${\rm X_6}$ Ile ${\rm X_8}\,{\rm X_9}\,{\rm X_{10}}\,{\rm X_{11}}$ Gly ${\rm X_{13}}\,{\rm X_{14}}\,{\rm X_{15}}$ Tyr ${\rm X_{17}}\,{\rm X_{18}}$ X₁₉X₂₀X₂₁X₂₂, wherein amino acid X₆ is Lys, Gln, Glu, Val, or Tyr, X₈ is Asn, Gly, Ser, Trp, or Tyr, X₉ is Ala, Pro or Ser, X₁₀ is Gly or Thr, X₁₁ is a peptide bond, Asp, Asn, Gly, or Ser, X_{13} is Asp, Asn, His, or Ser, X_{14} is Ser or Thr, X_{15} is Asn or Tyr, X₁₇ is Asn or Pro, X₁₈ is Ala, Asp, Gly, Gln, Glu, Pro, or Ser, X_{19} is Asn, Lys, Met, or Ser, X_{20} is Leu, Phe or Val, X_{21} is Lys, Met, or Gln, X_{22} is Asp, Gly or Ser. CDR_{H3} comprises the amino acid sequence $X_{23}\,X_{24}\,X_{25}\,X_{26}\,X_{27}\,X_{28}\,X_{29}\,X_{30}$ $X_{31} X_{32} X_{33} X_{34}$ Tyr, wherein amino acid X_{23} is Arg, Asn, Gln, or Glu, X₂₄ is Gly, Leu, Arg, or Tyr, X₂₅ is a peptide bond, Asp, or Gly, X_{26} is a peptide bond or Gly, X_{27} is a peptide bond or Tyr, X₂₈ is a peptide bond, Leu, or Tyr, X₂₉ is a peptide bond, Gly, Leu, Arg, or Val, $\rm X_{30}$ is a peptide bond, Asp, Gly, or Glu, X_{31} is a peptide bond, Asn, Arg, Ser, or Tyr, X_{32} is peptide bond, Ala, Gly, Ile, or Tyr, X_{33} is Met or Phe, X_{34} is Ala or Asp.

It is understood that the binding protein can comprise both the immunoglobulin light chain and the immunoglobulin heavy chain sequences or the fragments thereof, noted above. Furthermore, it is understood that the binding protein can be an intact antibody or an antigen binding fragment thereof, or a biosynthetic antibody site.

In certain embodiments, the CDR sequences of the immunoglobulin light chain and the immunoglobulin heavy chain are interposed with framework regions (FR).

In certain other embodiments, the CDR sequences of the immunoglobulin light chain and the immunoglobulin heavy chain are interposed between human or humanized framework regions.

In another aspect, the invention provides an isolated binding protein that specifically binds human HGF. The binding protein comprises: (a) an immunoglobulin light chain variable region comprising the structure CDR_{L1} - CDR_{L2} - CDR_{L3} and (b) immunoglobulin heavy chain variable region, wherein the immunoglobulin light chain variable region and 10 the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF. The CDR_{L1} comprises a sequence selected from the group consisting of SEQ ID NO. 8 (1A3), SEQ ID NO. 18 (2B8), SEQ ID NO. 28 (2F8), SEQ ID NO. 38 (3B6), SEQ ID NO. 48 15 (3D11), SEQ ID NO. 58 (1D3), SEQ ID NO. 68 (1F3), and SEQ ID NO. 78 (3A12). The CDR_{L2} comprises a sequence selected from the group consisting of SEQ ID NO. 9 (1A3), SEQ ID NO. 19 (2B8), SEQ ID NO. 29 (2F8), SEQ ID NO. 39 (3B6), SEO ID NO. 49 (3D11), SEO ID NO. 59 (1D3), SEO 20 ID NO. 69 (1F3), SEQ ID NO. 79 (3A12) and SEQ ID NO. 206 (LRMR2B8LC). The CDR_{L3} comprises a sequence selected from the group consisting of SEQ ID NO. 10 (1A3), SEQ ID NO. 20 (2B8), SEQ ID NO. 30 (2F8), SEQ ID NO. 40 (3B6), SEQ ID NO. 50 (3D11), SEQ ID NO. 60 (1D3), SEQ 25 ID NO. 70 (1F3), and SEQ ID NO. 80 (3A12). Throughout the specification and claims, the sequences denoted by a particular SEQ ID NO. are followed in parentheses by the antibody that was the origin of the particular sequence. By way of example, SEQ ID NO. 8 (1A3) indicates that the 30 sequence of SEQ ID NO. 8 is based upon the sequence present in antibody 1A3.

In one embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 8 (1A3), a 35 CDR_{L2} comprising the sequence of SEQ ID NO. 9 (1A3), and a CDR_{L3} comprising the sequence of SEQ ID NO. 10 (1A3).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR₁₁ comprising the sequence of SEQ ID NO. 18 (2B8), a 40 CDR_{L2} comprising the sequence of SEQ ID NO. 19 (2B8) or SEQ ID NO. 206 (LRMR2B8LC), and a CDR_{L3} comprising the sequence of SEQ ID NO. 20 (2B8).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a 45 CDR_{L1} comprising the sequence of SEQ ID NO. 28 (2F8), a CDR_{L2} comprising the sequence of SEQ ID NO. 29 (2F8), and a CDR_{L3} comprising the sequence of SEQ ID NO. 30 (2F8).

In another embodiment, the binding protein comprises an 50 immunoglobulin light chain variable region comprising a $CDR_{1,1}$ comprising the sequence of SEQ ID NO. 38 (3B6), a CDR_{L2} comprising the sequence of SEQ ID NO. 39 (3B6), and a CDR_{L3} comprising the sequence of SEQ ID NO. 40 (3B6).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 48 (3D11), a CDR_{L2} comprising the sequence of SEQ ID NO. 49 (3D11), and a CDR_{L3} comprising the sequence of SEQ ID NO. 50 60 (3D11).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 58 (1D3), a CDR_{L2} comprising the sequence of SEQ ID NO. 59 (1D3), 65 and a CDR_{L3} comprising the sequence of SEQ ID NO. 60 (1D3).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{11} comprising the sequence of SEQ ID NO. 68 (1F3), a CDR_{L2} comprising the sequence of SEQ ID NO. 69 (1F3), and a CDR_{L3} comprising the sequence of SEQ ID NO. 70 (1F3)

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{t1} comprising the sequence of SEQ ID NO. 78 (3A12), a CDR_{L2} comprising the sequence of SEQ ID NO. 79 (3A12), and a CDR_{L3} comprising the sequence of SEQ ID NO. 80 (3A12).

In each of the foregoing embodiments, the CDR_{L1} , CDR_{L2} , and CDR_{L3} sequences preferably are interposed between human or humanized immunoglobulin FRs. It is understood that the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1} - CDR_{H2} - CDR_{H3} , and (b) an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region together define a single binding site for binding human HGF. The CDR_{H1} comprises a sequence selected from the group consisting of SEQ ID NO. 5 (1A3), SEQ ID NO. 15 (2B8), SEQ ID NO. 25 (2F8), SEQ ID NO. 35 (3B6), SEQ ID NO. 45 (3D11), SEQ ID NO. 55 (1D3), SEQ ID NO. 65 (1F3), and SEQ ID NO. 75 (3A12); the CDR_{H2} comprises a sequence selected from the group consisting of SEQ ID NO. 6 (1A3), SEQ ID NO. 16 (2B8), SEQ ID NO. 26 (2F8), SEQ ID NO. 36 (3B6), SEQ ID NO. 46 (3D11), SEQ ID NO. 56 (1D3), SEQ ID NO. 66 (1F3), SEQ ID NO. 76 (3A12), SEQ ID NO. 202 (Hu2B8 Hv1f.1), SEQ ID NO. 203 (Hu2B8 Hv5a.1 or Hu2B8 Hv5-51.1), SEQ ID NO. 204 (LR2B8HC) and SEQ ID NO. 205 (LRMR2B8HC); and the CDR_{H3} comprises a sequence selected from the group consisting of SEQ ID NO. 7 (1A3), SEQ ID NO. 17 (2B8), SEQ ID NO. 27 (2F8), SEQ ID NO. 37 (3B6), SEQ ID NO. 47 (3D11), SEQ ID NO. 57 (1D3), SEQ ID NO. 67 (1F3), and SEQ ID NO. 77 (3A12).

In one embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 5 (1A3); a CDR_{H2} comprising the sequence of SEQ ID NO. 6 (1A3); and a CDR_{H3} comprising the sequence of SEQ ID NO. 7 (1A3).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 15 (2B8); a CDR_{H2} comprising the sequence of SEQ ID NO. 16 (2B8), SEQ ID NO. 202 (Hu2B8 Hv1f.1), SEQ ID NO. 203 (Hu2B8 Hv5a.1 or Hu2B8 Hv5-51.1), SEQ ID NO. 204 (LR2B8HC) or SEQ ID NO. 205 (LRMR2B8HC); and a CDR_{H3} comprising the sequence of SEQ ID NO. 17 (2B8).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 25 (2F8); a CDR_{H2} comprising the sequence of SEQ ID NO. 26 (2F8); and a CDR_{H3} comprising the sequence of SEQ ID NO. 27 (2F8).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the sequence of SEQ ID NO. 35 (3B6); a CDR_{H2} comprising the sequence of SEQ ID NO. 36 (3B6); and a CDR_{H3} comprising the sequence of SEQ ID NO. 37 (3B6).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 45 (3D11); a CDR_{H2} comprising the sequence of SEQ ID NO. 46 (3D11); and a CDR_{H3} comprising the sequence of SEQ ID NO. 47 5 (3D11).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 55 (1D3); a CDR_{H2} comprising the sequence of SEQ ID NO. 56 (1D3); 10 and a CDR_{H3} comprising the sequence of SEQ ID NO. 57 (1D3).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 65 (1F3); a 15 CDR_{H2} comprising the sequence of SEQ ID NO. 66 (1F3); and a CDR_{H3} comprising the sequence of SEQ ID NO. 67 (1F3).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a 20 CDR_{H1} comprising the sequence of SEQ ID NO. 75 (3A12); a CDR_{H2} comprising the sequence of SEQ ID NO. 76 (3A12); and a CDR_{H3} comprising the sequence of SEQ ID NO. 77 (3A12).

In each of the foregoing embodiments, the CDR_{H1} , 25 CDR_{H2} , and CDR_{H3} sequences preferably are interposed between human or humanized immunoglobulin FRs. It is understood that the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site. 30

In another aspect, the invention provides a binding protein that binds human HGF. The binding protein comprises an immunoglobulin heavy chain variable region selected from the group consisting of residues 20-141 of SEQ ID NO. 2 (1A3), residues 20-137 of SEQ ID NO. 12 (2B8), residues 35 20-137 of SEQ ID NO. 22 (2F8), residues 20-139 of SEQ ID NO. 32 (3B6), residues 20-132 of SEQ ID NO. 42 (3D11), residues 20-141 of SEQ ID NO. 52 (1D3), residues 20-141 of SEQ ID NO. 62 (1F3), and residues 20-141 of SEQ ID NO. 72 (3A12) and an immunoglobulin light chain variable region 40 selected from the group consisting of residues 21-127 of SEQ ID NO. 4 (1A3), residues 21-127 of SEQ ID NO. 14 (2B8), residues 20-131 of SEQ ID NO. 24 (2F8), residues 23-129 of SEQ ID NO. 34 (3B6), residues 23-128 of SEQ ID NO. 44 (3D11), residues 21-127 of SEQ ID NO. 54 (1D3), residues 45 21-127 of SEQ ID NO. 64 (1F3), and residues 21-127 of SEQ ID NO. 74 (3A12).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 2 50 (1A3), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 4 (1A3).

In one embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the 55 amino acid sequence of residues 20-137 of SEQ ID NO. 12 (2B8), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 14 (2B8).

In another embodiment, the binding protein comprises an 60 immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-137 of SEQ ID NO. 22 (2F8), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 20-131 of SEQ ID NO. 24 (2F8). 65

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-139 of SEQ ID NO. 32 (3B6), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 23-129 of SEQ ID NO. 34 (3B6).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-132 of SEQ ID NO. 42 (3D11), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 23-128 of SEQ ID NO. 44 (3D11).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 52 (1D3), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 54 (1D3).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 62 (1F3), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 64 (1F3).

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 72 (3A12), and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 74 (3A12).

In each of the foregoing embodiments, the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO. 173 (Hu2B8 Kv1-39.1 light chain variable region), SEQ ID NO. 179 (Hu2B8 Kv3-15.1 light chain variable region), SEQ ID NO. 193 (LR2B8LC light chain variable region), and SEQ ID NO. 199 (LRMR2B8LC light chain variable region); and (ii) an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO. 159 (Hu2B8 Hv1f.1 heavy chain variable region), SEQ ID NO. 165 (Hu2B8 Hv5a.1 heavy chain variable region), SEQ ID NO. 169 (Hu2B8 Hv5-51.1 heavy chain variable region), SEQ ID NO. 183 (LR2B8HC heavy chain variable region), and SEQ ID NO. 189 (LRMR2B8HC heavy chain variable region). The binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain selected from the group consisting of SEQ ID NO. 177 (Hu2B8 Kv1-39.1+ kappa constant (Km(3) allotype (allele 2)), SEQ ID NO. 181 (Hu2B8 Kv3-15.1+Kappa constant (Km(3) allotype (allele 2)), SEQ ID NO. 197 (LR2B8LC+Kappa constant (Km(3) allotype (allele 1)), and SEQ ID NO. 201 (LRMR2B8LC+ Kappa constant (Km(3) allotype (allele 1)); and (ii) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO. 163 (Hu2B8 Hv1f.1+IgG1 Constant (G1m(17, 1) allotype)), SEQ ID NO. 167 (Hu2B8 Hv5a.1+IgG1 Constant (G1m(17,1) allotype)), SEQ ID NO. 171 (Hu2B8 Hv5-51.1+IgG1 Constant (G1m(17,1) allotype)), SEQ ID NO. 187 (LR2B8HC+IgG1 Constant (G1m(3) allotype) (allele 1)), and SEQ ID NO. 191 (LRMR2B8HC+IgG1 Constant (G1m (3) allotype) (allele 1)). The binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

In another aspect, the invention provides an isolated binding protein that binds reduced human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region comprising three CDRs, and (ii) an immunoglobulin heavy chain variable region comprising three CDRs. The CDRs typically are interposed between FRs. The CDRs of the immunoglobulin light chain and the immunoglobulin heavy chain together define a binding site that binds reduced human HGF, for example, the α -chain of reduced HGF. Reduced HGF refers to HGF treated with an amount of reducing agent, for example, dithiothreitol (DTT), 2-mercaptoethanol, or glutathione sufficient to reduce the disulfide linkage between the α -chain and the β -chain. Exemplary concentrations include, for example, 100 mM DTT and 5% 2-mercaptoethanol.

In certain embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising at least one CDR selected from the group consisting of CDR_{L1} , CDR_{L2} and CDR_{L3} . Optionally, the binding protein comprises two CDRs, for example, CDR_{L1} and CDR_{L2} , or CDR_{L1}_{20} and CDR_{L3} , or CDR_{L1} and CDR_{L3} . Optionally, the binding protein comprises all three CDRs, i.e., CDR_{L1} , CDR_{L2} and CDR_{L3} . CDR_{L1} comprises the amino acid sequence $X_1 X_2$ Ser X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅, wherein amino acid X_1 is Arg or Lys, X_2 is Ala or Thr, X_4 is Glu or Gln, X_5 is Asn, Ser, or Asp, X₆ is Ile or Val, X₇ is Tyr, Asp, or Lys, X₈ is a peptide bond or Tyr, X_9 is a peptide bond or Asp, X_{10} is a peptide bond or Gly, X_{11} is a peptide bond or Asn, X_{12} is a peptide bond or Ser, X_{13} is Asn or Tyr, X_{14} is Ile or Leu, X_{15} is Ala, Asn, or Ser. CDR_{L2} comprises the amino acid sequence $\rm X_{16}\rm X_{17}\rm X_{18}\rm X_{19}$ Leu $\rm X_{21}\rm X_{22},$ wherein amino acid $\rm X_{16}$ is Ala, Asp, Val, or Arg, $\rm X_{17}$ is Ala or Val, $\rm X_{18}$ is Asn, Ser, or Thr, $\rm X_{19}$ is Arg, Asn, or His, X21 is Ala, Glu, Val, or Pro, X22 is Asp or Ser. CDR_{L3} comprises the amino acid sequence $X_{23} X_{24} X_{25}$ $X_{26}\,X_{27}\,X_{28}$ Pro X_{30} Thr, wherein amino acid X_{23} is Leu or 35 Gln, X_{24} is His or Gln, X_{25} is Phe, Ser, or Tyr, X_{26} is Asp, Ile, or Trp, X₂₇ is Gly or Glu, X₂₈ is Asp, Phe, or Thr, X₃₀ is Phe, Pro, or Tyr.

In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising at $_{40}$ least one CDR selected from the group consisting of CDR_{H1} , CDR_{H2} , and CDR_{H3} . Optionally, the binding protein comprises two CDRs, for example, CDR_{H1} and CDR_{H2} , or CDR_{H1} and CDR_{H3} , or CDR_{H1} and CDR_{H3} . Optionally, the binding protein comprises all three CDRs, i.e., CDR_{H1} , 45 CDR_{H2} and CDR_{H3} . CDR_{H1} comprises the amino acid sequence X1 Tyr X3 X4 X5, wherein amino acid X1 is Asp, Asn, Ser, or Thr, X₃ is Phe, Trp, or Tyr, X₄ is Ile or Met, X₅ is Asn, His, or Ser. CDR_{H2} comprises the amino acid sequence $\begin{array}{l} X_6 \ lle \ X_8 \ X_9 \ Gly \ X_{11} \ Gly \ X_{13} \ X_{14} \ X_{15} \ Tyr \ X_{17} \ X_{18} \ X_{19} \ X_{20} \ 50 \\ Lys \ X_{22}, wherein amino acid \ X_6 \ is \ Lys, \ Gln, \ or \ Tyr, \ X_8 \ is \ Gly, \end{array}$ Ser, or Tyr, X₉ is Pro or Ser, X₁₁ is Asp, Gly, or Ser, X₁₃ is Asp or Ser, $\rm X_{14}$ is Ser or Thr, $\rm X_{15}$ is Asn or Tyr, $\rm X_{17}$ is Asn or Pro, X18 is Ala, Asp, Gly, or Glu, X19 is Asn, Met, or Ser, X20 is Phe or Val, X_{22} is Asp or Gly. CDR_{H3} comprises the amino acid 55 sequence X₂₃ X₂₄ X₂₅ X₂₆ X₂₇ X₂₈ X₂₉ X₃₀ X₃₁ X₃₂ X₃₃ Asp Tyr, wherein amino acid $\rm X_{23}$ is Arg or Gln, $\rm X_{24}$ is Gly or Leu, X_{25} is Asp, Gly, or a peptide bond, X_{26} is Gly or a peptide bond, X27 is a peptide bond or Tyr, X28 is Leu, a peptide bond or Tyr, X_{29} is a Gly, Arg or Leu, X_{30} is Asp, Gly or Glu, X_{31} is $_{60}$ a Tyr, Arg or Asn, X₃₂ is Ala, Gly or Tyr, X₃₃ is Met or Phe.

It is understood that the binding protein can comprise both the immunoglobulin heavy chain and the immunoglobulin light chain sequences or the fragments thereof, noted above. Furthermore, it is understood that the binding protein can be an intact antibody or an antigen binding fragment thereof, or a biosynthetic antibody site.

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In certain embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising (i) a CDR_{11} having a sequence selected from the group consisting of SEQ ID NO. 8 (1A3), SEQ ID NO. 28 (2F8), SEQ ID NO. 38 (3B6), SEQ ID NO. 58 (1D3), and SEQ ID NO. 68 (1F3), (ii) a CDR_{L2} having a sequence selected from the group consisting of SEQ ID NO. 9 (1A3), SEQ ID NO. 29 (2F8), SEQ ID NO. 39 (3B6), SEQ ID NO. 59 (1D3), and SEQ ID NO. 69 (1F3), and (iii) a CDR_{L3} having a sequence selected from the group consisting of SEQ ID NO. 10 (1A3), SEQ ID NO. 30 (2F8), SEQ ID NO. 40 (3B6), SEQ ID NO. 60 (1D3), and SEQ ID NO. 70 (1F3). The CDR sequences can be interposed between human or humanized FRs. In other embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising an amino acid sequence selected from the group consisting of residues 21-127 of SEQ ID NO. 4 (1A3), residues 20-131 of SEQ ID NO. 24 (2F8), residues 23-129 of SEQ ID NO. 34 (3B6), residues 21-127 of SEQ ID NO. 54 (1D3), and residues 21-127 of SEO ID NO. 64 (1F3).

In certain other embodiments, the binding protein comprises an immunoglobulin heavy chain variable region comprising (i) a CDR_{H1} having a sequence selected from the group consisting of SEQ ID NO. 5 (1A3), SEQ ID NO. 25 (2F8), SEQ ID NO. 35 (3B6), SEQ ID NO. 55 (1D3), and SEQ ID NO. 65 (1F3), (ii) a CDR_{H2} having a sequence selected from the group consisting of SEQ ID NO. 6 (1A3), SEQ ID NO. 26 (2F8), SEQ ID NO. 36 (3B6), SEQ ID NO. 56 (1D3), and SEQ ID NO. 66 (1F3), and (iii) a CDR_{H3} having a sequence selected from the group consisting of SEQ ID NO. 7 (1A3), SEQ ID NO. 27 (2F8), SEQ ID NO. 37 (3B6), SEQ ID NO. 57 (1D3), and SEQ ID NO. 67 (1F3). The CDR sequences can be interposed between human or humanized FRs. In another embodiment, the immunoglobulin heavy chain variable region comprises an amino acid sequence selected from the group consisting of residues 20-141 of SEQ ID NO. 2 (1A3), residues 20-137 of SEQ ID NO. 22 (2F8), residues 20-139 of SEQ ID NO. 32 (3B6), residues 20-141 of SEQ ID NO. 52 (1D3), and residues 20-141 of SEQ ID NO. 62 (1F3).

In another aspect, the invention provides an isolated binding protein that binds human HGF and comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region. The isolated binding protein competes for binding to HGF with at least one reference antibody selected from the group consisting of (i) an antibody having an immunoglobulin light chain variable region of residues 20-131 of SEQ ID NO. 24 (2F8), and an immunoglobulin heavy chain variable region of residues 20-137 of SEQ ID NO. 22 (2F8), (ii) an antibody having an immunoglobulin light chain variable region of residues 23-129 of SEQ ID NO. 34 (3B6), and an immunoglobulin heavy chain variable region of residues 20-139 of SEQ ID NO. 32 (3B6), and (iii) an antibody having an immunoglobulin light chain variable region of residues 23-128 of SEQ ID NO. 44 (3D11), and an immunoglobulin heavy chain variable region of residues 20-132 of SEQ ID NO. 42 (3D11). Under certain circumstances, the binding protein binds the same epitope of HGF as one of the reference antibodies.

It is understood that each of the binding proteins discussed above can be an intact antibody, for example, a monoclonal antibody. Alternatively, the binding protein can be an antigen binding fragment of an antibody, or can be a biosynthetic antibody binding site. Antibody fragments include Fab, Fab', (Fab')₂ or Fv fragments. Techniques for making such antibody fragments are known to those skilled in the art. A number of biosynthetic antibody binding sites are known in the art and include, for example, single Fv or sFv molecules, described, for example, in U.S. Pat. No. 5,476,786. Other biosynthetic antibody binding sites include bispecific or bifunctional binding proteins, for example, bispecific or bifunctional antibodies, which are antibodies or antibody 5 fragments that bind at least two different antigens. For example, bispecific binding proteins can bind HGF, for example, human HGF, and another antigen of interest. Methods for making bispecific antibodies are known in art and, include, for example, by fusing hybridomas or by linking Fab' 10 fragments. See, e.g., Songsivilai et al. (1990) CLIN. EXP. IMMUNOL 79: 315-325; Kostelny et al. (1992) J. IMMUNOL 148: 1547-1553.

The binding proteins of the invention can bind hHGF containing a cysteine to arginine substitution at position 561 or a 15 glycine to glutamate substitution at position 555.

In another aspect, the invention provides an isolated binding protein that binds human HGF with a k_d of $4.0 \times 10^{-5} \text{ s}^{-1}$ or lower, $3.0 \times 10^{-5} \text{ s}^{-1}$ or lower, or $2.0 \times 10^{-5} \text{ s}^{-1}$ or lower. The isolated binding proteins can bind human HGF with a k_d from 20 $5.0 \times 10^{-5} \text{ s}^{-1}$ to $0.5 \times 10^{-5} \text{ s}^{-1}$, or from $4.0 \times 10^{-5} \text{ s}^{-1}$ to $1.0 \times$ 10^{-5} s^{-1} , or from $3.0 \times 10^{-5} \text{ s}^{-1}$ to $1.5 \times 10^{-5} \text{ s}^{-1}$. In another aspect, the invention provides an isolated binding protein that binds human HGF with a K_D of 100 pM or lower, or 20 pM or lower, or 10 pM or lower, or 5 pM or lower. The isolated 25 binding proteins can bind human HGF with a K_D from 100 pM to 5 pM, or from 20 pM to 5 pM, or from 15 pM to 10 pM, or from 20 pM to 10 pM, or from 15 pM to 5 pM. Unless otherwise specified, K_D values are determined by the methods, and under the conditions, described in Example 6. 30

In another aspect, the invention provides an isolated binding protein that binds human HGF, wherein the antibody binds to human HGF with lower K_D at 37° C. than at 25° C. The binding protein binding optionally binds human HGF with a K_D less than 5 pM at 37° C.

In other aspects and embodiments, the binding proteins can inhibit hHGF from binding to c-Met. For example, the binding proteins can have an IC₅₀ (concentration at 50% of maximum inhibition) of at least about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 nM when assayed using the protocol described in ⁴⁰ Example 7(a). In certain other embodiments, the binding proteins can neutralize HGF BrdU incorporation in 4 MBr-5 cells (ATCC, Catalog No. CCL208) using the method described in Example 7(b).

The binding proteins have an IC_{50} of 50 nM or lower, ⁴⁵ preferably 45, 40, 35, 30, 25, 20, 15, 10, 5, 1, 0.5 nM or lower, when assayed using the protocol described in Example 7(b). In certain other embodiments, the binding proteins can be used to inhibit HGF stimulated c-Met phosphorylation in PC-3 cells (ATCC, Manassus, Va. Catalog No. CRL-1435) ⁵⁰ using the assay described in Example 9. The binding proteins inhibit HGF-stimulated (1.25 nM) c-Met phosphorylation in PC-3 cells with an IC_{50} of 2 nM or less (Table 8), using the assay described in Example 9.

II—Production of Binding Proteins

Binding proteins of the invention can be produced in various ways using approaches know in the art. For example, DNA molecules encoding light chain variable regions and heavy chain variable regions can be chemically synthesized, 60 using a commercial synthesizer and sequence information provided herein. Such synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, e.g., constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs encoding the desired binding proteins. Production of defined gene constructs is within routine skill in the art.

Alternatively, the sequences provided herein can be cloned out of hybridomas by conventional hybridization techniques or PCR techniques, using synthetic nucleic acid probes whose sequences are based on sequence information provided herein or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells. Production and use of such probes is within ordinary skill in the art.

The nucleic acids encoding the desired binding proteins can be introduced (ligated) into expression vectors, which can be introduced into a host cell via standard transfection or transformation techniques known in the art. Exemplary host cells include, for example, *E. coli* cells, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma cells that do not otherwise produce immunoglobulin protein. Transfected host cells can be grown under conditions that permit the host cells to express the genes of interest, for example, the genes that encode the immunoglobulin light or heavy chain variable regions. The resulting expression products can be harvested using techniques known in the art.

The particular expression and purification conditions will vary depending upon what expression system is employed. For example, if the gene is to be expressed in *E. coli*, it is first cloned into an expression vector. This is accomplished by positioning the engineered gene downstream from a suitable bacterial promoter, e.g., Trp or Tac, and a signal sequence, e.g., a sequence encoding fragment B of protein A (FB). The resulting expressed fusion protein typically accumulates in refractile or inclusion bodies in the cytoplasm of the cells, and may be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the expressed proteins refolded and cleaved by the methods already established for many other recombinant proteins.

If the engineered gene is to be expressed in eukaryotic host cells, for example, myeloma cells or CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, immunoglobulin enhancers, and various introns. This expression vector optionally can contain sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be transfected into myeloma cells or CHO cells using established transfection protocols. Such transfected cells can express V_L or V_H fragments, V_L - V_H heterodimers, V_H - V_L or V_L - V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a protein domain having another function (e.g., cytotoxicity).

III—Modifications to the Binding Proteins

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It is understood that the binding proteins can be modified to optimize performance depending upon the intended use of the binding proteins. For example, when the binding protein is being used as a therapeutic agent, the binding protein can be modified to reduce its immunogenicity in the intended recipient. Alternatively or in addition, the binding protein can be fused or coupled to another protein or peptide, for example, a growth factor, cytokine, or cytotoxin. Such modifications can be achieved by using routine gene manipulation techniques known in the art.

Various techniques for reducing the antigenicity of antibodies and antibody fragments are known in the art. These techniques can be used to reduce or eliminate the antigenicity of the binding proteins of the invention. For example, when the binding proteins are to be administered to a human, the

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binding proteins preferably are engineered to reduce their antigenicity in humans. This process often is referred to as humanization. Preferably, the humanized binding proteins have the same or substantially the same affinity for the antigen as the original non-humanized binding protein it was derived 5 from.

In one well known humanization approach, chimeric proteins are created in which immunoglobulin constant regions of antibodies from one species, e.g., mouse, are replaced with immunoglobulin constant regions from a second, different species, e.g., a human. In this example, the resulting antibody is a mouse-human chimera, where the human constant region sequences, in principle, are less immunogenic than the counterpart murine sequences. This type of antibody engineering 15 is described, for example, Morrison, et al. (1984) PROC. NAT. ACAD. Sci. 81: 6851-6855, Neuberger et al. (1984) NATURE 312: 604-608; U.S. Pat. No. 6,893,625 (Robinson); U.S. Pat. No. 5,500,362 (Robinson); and U.S. Pat. No. 4,816,567 (Cabilly).

In another approach, known as CDR grafting, the CDRs of 20 the light and heavy chain variable regions of an antibody of interest are grafted into frameworks (FRs) from another species. For example, murine CDRs can be grafted into human FR sequences. In some embodiments, the CDRs of the light 25 and heavy chain variable regions of an anti-HGF antibody are grafted into human FRs or consensus human FRs. In order to create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described, for example, in U.S. Pat. No. 7,022,500 (Queen); U.S. Pat. NO. 6,982,321 (Winter); U.S. Pat. No. 6,180,370 (Queen); U.S. Pat. No. 6,054,297 (Carter); U.S. Pat. No. 5,693,762 (Queen); U.S. Pat. No. 5,859,205 (Adair); U.S. Pat. No. 5,693,761 (Queen); U.S. Pat. No. 5,565,332 (Hoogenboom); U.S. Pat. No. 5,585,089 (Queen); U.S. Pat. No. 5,530,101 (Queen); U.S. Pat. NO. Jones et al. (1986) NATURE 321: 522-525; Riechmann et al. (1988) NATURE 332: 323-327; Verhoeyen et al. (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS Lett 430: 92-94.

In an approach called "superhumanization," antibodies in which human immunogenicity is reduced or eliminated are created by an alternative form of grafting. In superhumanization, human FR sequences are chosen from a set of human germline genes based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. This approach is described, for example, in U.S. Pat. No. 6,881,557 (Foote) and in Tan et al. (2002) J. IMMUNOL 169: 1119-1125.

Other approaches to reduce immunogenicity include, tech- 50 niques are known as "reshaping," "hyperchimerization," or "veneering/resurfacing" to produce humanized antibodies. See, e.g., Vaswami et al. (1998) Annals of Allergy, ASTHMA, & IMMUNOL. 81: 105; Roguska et al. (1996) PROT. ENGINEER 9: 895-904; and U.S. Pat. No. 6,072,035 (Hardman). 55 IV—Use of Binding Proteins In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, for example, in U.S. Pat. No. 5,639,641 (Peder- 60 sen)

One exemplary approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMABTM technology (Vaccinex, Inc., Rochester, N.Y.), which involves a vaccinia virus-based vector to express 65 antibodies in mammalian cells. High levels of combinatorial diversity of immunoglobulin heavy and light chains are said

to be produced. See, e.g., U.S. Pat. Nos. 6,706,477 (Zauderer); U.S. Pat. NO. 6,800,442 (Zauderer); and U.S. Pat. No. 6,872,518 (Zauderer).

Another exemplary approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, Calif.). This technology involves the use of a proprietary human "acceptor" library to produce an "epitope focused" library for antibody selection.

Another exemplary approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERINGTM (HETM) technology, which is practiced commercially by XOMA (US) LLC. See, e.g., International Application Publication No. WO 93/11794 and U.S. Pat. Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869, 619.

Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of a binding protein of interest.

In addition, it is possible to create fully human antibodies in mice. In this approach, human antibodies are prepared using a transgenic mouse in which the mouse's antibodyproducing genes have been replaced by a substantial portion of the human antibody producing genes. Such mice produce human immunoglobulin instead of murine immunoglobulin molecules. See, e.g., WO 98/24893 (Jacobovitz et al.) and Mendez et al. (1997) NATURE GENETICS 15: 146-156. Fully human anti-HGF monoclonal antibodies can be produced using the following approach. Transgenic mice containing human immunoglobulin genes are immunized with the antigen of interest, e.g., HGF. Lymphatic cells from the mice then are obtained from the mice, which are then fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. The hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to HGF.

Binding proteins of the invention can be conjugated with other molecules, depending upon their intended use. For example, if the binding protein is going to be used as a therapeutic, then the binding protein can be conjugated with another agent, for example, an effector molecule that modulates or otherwise promotes the therapy. To the extent that the effector is non-protein based agent, for example, a small molecule drug, a radiolabel or toxin, then, the agent can be chemically coupled to the binding protein using standard in vitro coupling chemistries. If, on the other hand, the effector molecule is a protein or peptide, for example, an enzyme, receptor, toxin, growth factor, cytokine or other immunomodulator, then the binding protein can either be chemically coupled to the effector using in vitro coupling chemistries or can be coupled to the effector as a fusion protein. Fusion proteins can be constructed and expressed using the techniques similar to those discussed in section II.

The binding proteins described herein can be used as a diagnostic agent or a therapeutic agent.

(1) Therapeutic Applications

Because the binding proteins of the invention neutralize the activity of HGF, they can be used in various therapeutic applications. For example, certain binding proteins of the invention are useful in the prevention or treatment of hyperproliferative diseases or disorders, e.g., various forms of cancer.

The binding proteins can be used to inhibit or reduce the proliferation of tumor cells. In such an approach, the tumor cells are exposed to a therapeutically effective amount of the

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binding protein so as to inhibit or reduce proliferation of the tumor cell. In certain embodiments, the binding proteins inhibit tumor cell proliferation by at least 50%, 60%, 70%, 80%, 90%, 95% or 100%.

In certain embodiments, the binding protein is used to inhibit or reduce proliferation of a tumor cell wherein the binding protein reduces the ability of hHGF to bind to c-Met. In other embodiments, the binding protein is used to inhibit or reduce the proliferation of a tumor cell even when the binding protein binds hHGF but does not substantially inhibit hHGF binding to c-Met, as shown by antibody 3B6 in Tables 5 and 6.

In addition, the binding protein can be used to inhibit, or slow down tumor growth or development in a mammal. In such a method, an effective amount of the binding protein is administered to the mammal so as to inhibit or slow down tumor growth in the mammal. Accordingly, the binding proteins can be used to treat tumors, for example, in a mammal. The method comprises administering to the mammal a thera-20 peutically effective amount of the binding protein. The binding protein can be administered alone or in combination with another pharmaceutically active molecule, so as to treat the tumor.

It is contemplated that the binding proteins of the invention can be used in the treatment of a variety of HGF responsive disorders, including, for example, HGF responsive tumor cells in lung cancer, breast cancer, colon cancer, prostate cancer, ovarian cancer, head and neck cancer, ovarian cancer, multiple myeloma, liver cancer, gastric cancer, esophageal cancer, kidney cancer, nasopharyngeal cancer, pancreatic cancer, mesothelioma, melanoma and glioblastoma.

As used herein, "treat, "treating" and "treatment" refer to the treatment of a disease-state in a mammal, particularly in a human, and include: (a) preventing the disease-state from 35 occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, i.e., arresting its development; and/or (c) relieving the disease-state, i.e., causing regression of the disease state.

Generally, a therapeutically effective amount of active component will be in the range of from about 0.1 mg/kg to about 100 mg/kg, optionally from about 1 mg/kg to about 100 mg/kg, optionally from about 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the 45 type and extent of disease or indication to be treated, the overall health status of the particular patient, the relative biological efficacy of the binding protein delivered, the formulation of the binding protein, the presence and types of excipients in the formulation, and the route of administration. 50 The initial dosage administered may be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue level, or the initial dosage may be smaller than the optimum and the daily dosage may be progressively increased during the course of treatment depending on the 55 particular situation. Human dosage can be optimized, e.g., in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount and the disease condition being treated. Exemplary 60 dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, e.g., intravenous infusion. Formulation of monoclonal antibody-based drugs is within ordinary skill in the art. In some embodiments of the invention, the binding protein, 65 e.g., monoclonal antibody, is lyophilized and reconstituted in buffered saline at the time of administration.

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The binding proteins may be administered either alone or in combination with other pharmaceutically active ingredients. The other active ingredients, e.g., immunomodulators, can be administered together with the binding protein, or can be administered before or after the binding protein.

Formulations containing the binding proteins for therapeutic use, typically include the binding proteins combined with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients, that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers, in this regard, are intended to include any and all buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

The formulations can be conveniently presented in a dosage unit form and can be prepared by any suitable method, including any of the methods well known in the pharmacy art. A pharmaceutical composition of the invention should be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral administration or non-parenteral administration, for example, intravenous, intradermal, inhalation, transdermal (topical), transmucosal, and rectal administration. Useful solutions for oral or parenteral administration can be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences, 18th ed. (Mack Publishing Company, 1990).

Formulations suitable for oral administration can be in the form of: discrete units such as injectables, capsules, gelatin capsules, sachets, tablets, troches, or lozenges, each containing a predetermined amount of the binding protein; a powder or granular composition; a solution or a suspension in an aqueous liquid or non-aqueous liquid; or an oil-in-water emulsion or a water-in-oil emulsion.

Formulations suitable for parenteral administration include, for example, the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

In general, compositions suitable for injectable use include aqueous solutions (where water soluble) or dispersions and powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid 5 polyetheylene glycol), and suitable mixtures thereof.

Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be con- 10 ducted prior to or following lyophilization and reconstitution. Once the pharmaceutical composition has been formulated, it can be stored, for example, in vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder.

(2) Diagnostic Applications

Whenever the binding proteins are used for diagnostic purposes, either in vitro or in vivo, the binding proteins typically are labeled either directly or indirectly with a detectable moiety. The detectable moiety can be any moiety which is capable of producing, either directly or indirectly, a detect- 20 able signal. For example, the detectable moiety may be a radioisotope, such as ³Hydrogen (³H), ¹⁴Carbon (¹⁴C), ³²Phosphorus (³²P), ³⁵Sulfur (³⁵S), or ¹²⁵Iodine (¹²⁵I); a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; an enzyme, such as 25 alkaline phosphatase, beta-galactosidase, or horseradish peroxidase; a spin probe, such as a spin label; or a colored particle, for example, a latex or gold particle. It is understood that the binding protein can be conjugated to the detectable moiety using a number of approaches known in the art, for 30 example, as described in Hunter et al. (1962) NATURE 144: 945; David et al. (1974) BIOCHEMISTRY 13: 1014; Pain et al. (1981) J. IMMUNOL. METH. 40: 219; and Nygren (1982) J. HIS-TOCHEM. AND CYTOCHEM. 30: 407. The labels may be detected, e.g., visually or with the aid of a spectrophotometer or other 35 detector.

The binding proteins can be employed in a wide range of immunoassay techniques available in the art. Exemplary immunoassays include, for example, sandwich immunoassays, competitive immunoassays, immunohistochemical pro- 40 cedures.

In a sandwich immunoassay, two antibodies that bind an analyte or antigen of interest are used, e.g., one immobilized onto a solid support, and one free in solution and labeled with a detectable moiety. When a sample containing the antigen is 45 introduced into this system, the antigen binds to both the immobilized antibody and the labeled antibody, to form a "sandwich" immune complex on the surface of the support. The complexed protein is detected by washing away nonbound sample components and excess labeled antibody, and 50 measuring the amount of labeled antibody complexed to protein on the support's surface. Alternatively, the antibody free in solution can be detected by a third antibody labeled with a detectable moiety which binds the free antibody. A detailed review of immunological assay design, theory and protocols 55 can be found in numerous texts, including Butt, ed., (1984) PRACTICAL IMMUNOLOGY, Marcel Dekker, New York; Harlow et al. eds. (1988) ANTIBODIES, A LABORATORY APPROACH, Cold Spring Harbor Laboratory; and Diamandis et al., eds. (1996) IMMUNOASSAY, Academic Press, Boston. 60

It is contemplated that the labeled binding proteins are useful as in vivo imaging agents, whereby the binding proteins can target the imaging agents to particular tissues of interest in the recipient. A preferred remotely detectable moiety for in vivo imaging includes the radioactive atom Tech- 65 netium^{-99m} (^{99m}Tc), a gamma emitter with a half-life of about six hours. Non-radioactive moieties also useful in in vivo

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imaging include nitroxide spin labels as well as lanthanide and transition metal ions all of which induce proton relaxation in situ. In addition to immunoimaging, the complexed radioactive moieties may be used in standard radioimmunotherapy protocols to destroy the targeted cell. Preferred nucleotides for high dose radioimmunotherapy include the radioactive atoms ⁹⁰Yttrium (⁹⁰Yt), ¹³¹Iodine (¹³¹I) and ¹¹¹Indium $(^{111}$ In). The binding protein can be labeled with 131I, 111 In and ^{99m}TC using coupling techniques known in the imaging arts. Similarly, procedures for preparing and administering the imaging agent as well as capturing and processing images are well known in the imaging art and so are not discussed in detail herein. Similarly, methods for performing antibodybased immunotherapies are well known in the art. See, for example, U.S. Pat. No. 5,534,254.

Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Except where indicated otherwise, the order of steps or order for performing certain actions are immaterial so long as the invention remains operable. Moreover, unless otherwise noted, two or more steps or actions may be conducted simultaneously.

EXAMPLES

The following Examples discuss the production and characterization of a number of anti-hHGF monoclonal antibodies

Example 1

Production of Anti-hHGF Monoclonal Antibodies

This Example describes the production of a number of anti-hHGF monoclonal antibodies.

Immunizations, fusions, and primary screens were conducted at MBS Inc. (Portland, Me.), following the Repetitive Immunization Multiple Sites (RIMMS) protocol. Five AJ mice and Five Balb/c mice were immunized with recombinant human HGF (R&D Systems, Minneapolis, Minn.; Catalog No. 294-HGN-025). Two mice with sera displaying highest anti-HGF activity by Enzyme Linked Immunosorbent Assay (ELISA) were chosen for subsequent fusion. Spleens and lymph nodes from the appropriate mice were harvested. B-cells then were harvested and fused with an myeloma line. Fusion products were serially diluted on one or more plates to near clonality. Supernatants from the resulting fusions were screened for their binding to hHGF by ELISA. Supernatants identified as containing antibodies to HGF were further characterized by in vitro functional testing as discussed in the following examples. A panel of hybridomas was selected and the hybridomas were subcloned and expanded. The monoclonal antibodies then were purified by affinity chromatography on Protein A/G resin under standard conditions.

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Example 2

Sequence Analysis of Anti-hHGF Monoclonal Antibodies

This Example describes isotype and sequence analyses of the anti-hHGF monoclonal antibodies produced in Example 1.

a. Determination of HGF Murine Monoclonal Antibody Isotypes

The light-chain type and heavy chain isotype of each monoclonal antibody were determined using the IsoStrip Mouse Monoclonal Antibody Isotyping Kit in accordance the manufacturer's instructions (Roche Applied Science).

All the antibodies were determined to contain a Kappa immunoglobulin light chain and an IgG1 immunoglobulin heavy chain.

b. Determination of Nucleotide Sequences Encoding Immunoglobulin Heavy and Light Chain Variable Regions

The variable regions of the Kappa and Heavy (IgG1) immunoglobulin chains were amplified by PCR (Polymerase Chain Reaction) using the Expand High-Fidelity PCR System (Roche Applied Science) according to the manufactur- 35 er's instructions. Heavy chain variable regions were amplified with the 5' oligonucleotide primer mix Universal Primer of 5' ctaatacgactcactatagggcaag-Mix A (mix cagtggtatcaacgcagagt 3' (SEQ ID NO. 87) and 5' ctaatacgactcactataggge 3'(SEQ ID NO. 88)) and a 3' IgG1 Constant 40 Region specific primer, either 5' tatgcaaggcttacaaccaca 3' (SEQ ID NO. 89) or 5' gccagtggatagacagatgggggtgtcg 3' (SEQ ID NO. 90). Kappa chain variable regions were amplified with the 5' oligonucleotide primer mix Universal Primer Mix A and a 3' Kappa Constant Region specific primer, either 45 5' ctcattcctgttgaagctcttgacaat 3' (SEQ ID NO. 91) or 5' cgactgaggcacctccagatgtt 3' (SEQ ID NO. 92).

Individual PCR products were fractionated by agarose gel electrophoresis and purified using the Qiaquick Gel Purification kit according to the manufacturer's instructions 50 (Qiagen). The PCR products were subsequently cloned into the pCR2.1 TOPO plasmid using the topoisomerase based cloning kit TOPO TA Cloning® Kit (with pCR®2.1-TOPO® vector) according to the manufacturer's instructions (Invitrogen, Carlsbad, Calif.) and transformed into DH5 bacteria 55 using standard transformation techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using T7 (5' TAATACGACTCACTATAGGG 3') (SEQ ID NO. 93), M13 Forward (5' GTAAAACGACGGCCAGT 3') (SEQ ID NO. 94), and M13 Reverse primers (5' CAGGAAACAGC- 60 TATGACC 3') (SEQ ID NO. 95) by Agencourt Bioscience using standard dideoxy DNA sequencing methods to identify the sequence of the variable region sequences. The sequences were analyzed using Vector NTI software (Invitrogen, Carlsbad, Calif.) and the IMGT/V-Quest webserver (http://imgt-65 .cines.fr/textes/vquest) to identify and confirm variable region sequences.

c. Determination of Nucleotide Sequences Encoding Immunoglobulin Heavy and Light Chain Constant Region Sequences for 1A3, 1D3, 1F3, and 2B8 Kappa and IgG1 Chains

Full Length cDNAs for the 1A3, 1D3, and 1F3 IgG1 chains were PCR amplified from the cDNA created above using the forward primer 5' ggggacaagtttgtacaaaaaagcaggctgccacc atgaactttgggctcagattgatttcc 3' (start codon underlined) (SEQ ID NO. 96) and the reverse primer 5' ggggaccactttgtacaa-gaaagctgggttcatttaccaggagagtgggagagg 3' (stop codon underlined) (SEQ ID NO. 97). Full Length cDNA for the 2B8 IgG1 chain was amplified from the cDNA created above using the forward primer 5' ggggacaagtttgtacaaaaaagcaggctgccacc atggatggagctatacatcctttt 3' (start codon underlined) (SEQ ID NO. 98) and reverse primer 5' ggggaccactttgtacaa-gaaagctgggttcatttaccaggagagtggagag 3' (stop codon underlined) (SEQ ID NO. 98) and reverse primer 5' ggggaccactttgtacaa-gaaagctgggttcatttaccaggagagtggagag 3' (stop codon underlined) (SEQ ID NO. 98) and reverse primer 5' ggggaccactttgtacaa-gaaagctgggttcatttaccaggagagtggagag 3' (stop codon underlined) (SEQ ID NO. 99).

Full Length cDNA for the 2B8 Kappa Chain was amplified using the forward primer 5' ggggacaagtttgtacaaaaagcaggctgccaccatgaatcacagactctggtcttcata 3' (start codon underlined) (SEQ ID NO. 100) and the reverse primer 5' ggggaccactttgtacaagaaagctgggtctaacactcattcctgttgaagctc 3' (stop codon underlined) (SEQ ID NO. 101). PCR fragments were subcloned into pDONR221 (Invitrogen, Carlsbad, Calif.) by Gateway BP recombination reaction (Invitrogen, Carlsbad, Calif.) and sequenced by Agencourt Bioscience using standard dideoxy DNA sequencing methods to identify the sequence of the constant region and further confirm variable region sequences.

d. Sequence Analysis

Variable Regions (normal text) were identified using IMGT/V-QUEST webserver software (http://imgt.cines.fr/ textes/vquest/). Signal Peptide sequences were predicted based on identification of the in frame start codon (ATG) that was upstream of the identified Variable Region. Signal Peptide sequences were identified and are underlined below.

The last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region junction. This nucleotide is included in the variable region cause it is part of that exon. Amino acid sequences of the constant regions listed below include the translation of this junction codon.

In order to create the complete heavy or kappa chain antibody sequences, the variable region sequences noted below are combined with their respective constant region sequences (the signal sequences are underlined).

- 1A3 Heavy Chain Variable Region (SEQ ID NO. 1)
 1 <u>atgaactttg qqctcaqatt qattttcctt qtccttqttt</u> <u>taaaaqgtgt gaagtgtgaa</u>
- 61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
- 121 tgtgcagcct ctgaattcac tttcagtaac tattacatgt cttgggttcg ccagactcca
- 181 gagaagagge tgcagtgggt cgcatacatt agteetggtg gtggtagete etaetateea
- 241 gccagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
- 301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtqcaag acaaggggat

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- 361 ggttactacg gggactatgc tatggactac tggggtcaag qaacctcaqt caccqtctcc
- 421 tcag
- (2) 1A3 Kappa Light Chain Variable Region (SEQ ID NO. 3)
 - 1 <u>atgagtgtgc ccactcaggt cctggggttg ctgctgctgt</u> <u>ggcttacaga tgccagatgt</u>
 - 61 gacatecaga tgacteagte tecageetee etatetgttt ctgtgggaga aactgteace
- 121 atcacatgtc gagcaagtga gaatatttat agtaatttag catggtatca gcagaaacag
- 181 ggaaaatete etcageteet ggtetatget geaacaaaet tageagatgg tgtgeeatea
- 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
- 301 gaagattttg ggacttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
- 361 gggaccaagc tggaaataaa ac
- (3) 2B8 Heavy Chain Variable Region (SEQ ID NO.11)
 - 1 <u>atgggatgga gctatatcat cctctttttg gtagcaacag</u> <u>ctacagatgt ccactcc</u>cag
 - 61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
- 121 tgcaaggett etggetacae etteaceaee taetggatge aetgggtgaa teagaggeet
- 181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
- 241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg
- 301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
- 361 ggtagcatet ttgactaetg gggeeaagge accaetetea caqteteete aq
- (4) 2B8 Kappa Light Chain Variable Region (SEQ ID NO. 13)
 - 1 <u>atggaatcac agactctggt cttcatatcc atactgctct</u> <u>ggttatatgg tgctgatggg</u>
 - 61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
- 121 ttgagetgea aggeeagtga gaatgtggtt tettatgtat eetggtatea acagaaacea
- 181 gcgcagtete etaaactget gatataeggg geateeaace ggaacaetgg ggteeeegat
- 241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
- 301 gaagacettg cagattatea etgtgggeag agttacaaet ateegtacae gtteggaggg
- 361 gggaccaggc tggaaataaa ac
- (5) 2F8 Heavy Chain Variable Region (SEQ ID NO.
- 21)
 - 1 <u>atggaatgga getgggtett tetetteete etgteagtaa</u> <u>etgeaggtgt ceaetge</u>eag

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- 61 gtccagctga agcagtctgg agctgagctg gtgaggcctg ggacttcagt gaagatgtcc
- 121 tgcaaggett etggetaeae etteaetaee taetatae aetgggtgaa teagaggeet
- 181 ggacagggcc ttgagtggat tggaaagatt ggtcctggaa gtggtagtac ttactacaat
- 241 gagatgttca aagacaaggc cacattgact gtagacacat cctccagcac agcctacatg
- 301 cageteagea geetgacate tgaegaetet geggtetatt tetgtgeaag aaggggaetg
- 361 ggacgtggct ttgactactg gggccaaggc accactctca cagteteete ag
- (6) 2F8 Kappa Light Chain Variable Region (SEQ ID $20~\mbox{NO. 23})$
 - 1 <u>atggagacag acacaateet getatgggtg etgetgetet</u> <u>gggtteeagg etceaetggt</u>
 - 61 gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc
 - 121 atctcctgca aggccagcca aagtgttgat tatgatggta atagttatat caactggtac
 - 181 caacagaaac caggacagcc acccaaagtc ctcatctatg ttgcatccaa tctagaatct
 - 241 gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat
 - 301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc aaagtattga ggatcctccc
 - 361 acgttcggtg ctgggaccaa gctggagctg aaac
 - (7) 3B6 Heavy Chain Variable Region (SEQ ID NO.
 - 1 <u>atggaatggc cttgtatctt tctcttcctc ctgtcagtaa</u> ctgaaggtqt ccactcccag
 - 61 gttcagctgc agcagtctgg ggctgaactg gtgaggcctg ggtcctcagt gaagatttcc
 - 121 tgcaaggett etggetatgt atteagtage taetggatga aetgggtgaa geagaggeet
 - 181 ggacagggtc ttgagtggat tggacagatt tatcctggag atggtgatag taactacaat
 - 241 ggaaacttca agggtaaagc cacactgact gcagacaaat cctccagtac agcctacatg
 - 301 cageteagea geetaacate tgaggaetet geggtetatt tetgtgeate eeageteggg
 - 361 ctacgtgaga actactttga ctactggggc caaggcacca ctctcacagt ctcctcag
- 60 (8) 3B6 Kappa Light Chain Variable Region (2 possible ATG start codons (uppercase)) (SEQ ID NO. 33)
 - 1 <u>ATGgacATGa ggacccctgc tcagtttctt ggaatcttgt</u> <u>tgctctggtt tccaggtatc</u>
 - 61 <u>aaatqt</u>gaca tcaagatgac ccagtctcca tcttccatgt atgcatctct aggagagaga

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- 121 gtcacaatca cttgcaaggc gagtcaggac attaaaagct atttaaqctg gttccagcag
- 181 aaaccaggga aatctcctaa gaccctgatc tatcgtgtaa acagattggt agatggggtc
- 241 ccatcaaggt tcagtggcag tggatctggg caagattett eteteaceat caccageetg
- 301 gagaatgaag atatgggaat ttattattgt ctacagtatg atgagtttcc gttcacgttc
- (9) 3D11 Heavy Chain Variable Region (SEQ ID NO. 41)
- 1 <u>atggetgtec eggtgetgtt cetetgeetg gttgeattte</u> caagetgtgt cetgteeeag
- 61 gtacagetga aggagteagg acetggeetg gtggegeeet caeaqageet gteeateact
- 121 tgcactgtct ctgggttttc attaaccagc tatagtttac actgggttcg ccagcctcca
- 181 ggaaagggtc tggaatggct gggagtaata tgggctggtg gaaacacaaa ttataattcg
- 241 teteteatgt eeagaetgae eateaggaaa gaeaaeteea agageeaagt tttettaaaa
- 301 atgaacagtc tgcaaactga tgacacagcc atgtactact gtgccagaga gaggtttgct
- 361 tactggggcc aagggactct ggtcactgtc tctgcag
- (10) 3D11 Kappa Light Chain Variable Region (SEQ ID NO. 43)
 - 1 <u>atggattttc aagtgcagat tttcagcttc ctgctaatca</u> gtgcctcagt caaaatatcc
 - 61 <u>aqaqqa</u>caaa ttgttctcac ccagtctcca gcaatcatgt ctgcatatcc aggggagaag
- 121 gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgcactggta ccagcagaag
- 181 teaggeacet eccecaaaag atggatttat gacacateea aaetggette tggagteeet
- 241 getegettea gtggeagtgg gtetgggaee tettaeteee teacaateag tagtatggag
- 301 getgaagatg etgecaetta ttaetgecag eagtggagta gtaacceaet eaegtteggt
- 361 gctgggacca agctggagct gaaac

(11) 1D3 Heavy Chain Variable Region (SEQ ID NO. 51)

- 1 <u>atgaactttg ggeteagatt gatttteett gteettgttt</u> <u>taaaaggtgt gaagtgtg</u>aa
- 61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
- 121 tgtgcagcct ctggattcac tttcagtgac tattacatgt cttgggttcg ccagactcca
- 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
- 241 gacagtgtga agggtcgatt caccatetee cgagacaatg ccaagaacae cetgtaeetg
- 301 caaatgagca gtctgaagtc tgaggacaca gccatatatt actgtgtgag acaaggggat

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361 ggttattacg gggactatgc taiggactac tggggtcaag gaacctcagt catcgtctcc

421 tcag

- (12) 1D3 Kappa Light Chain Variable Region (SEQ ID NO. 53)
- 1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt
 ggcttacaga tgtcagatgt
- 61 gacatecaga tgaeteagte tecageetee etatetgtat etgtgggaga aaetgteace
- 121 atcacatgtc gaacaagtga gaatatttac agtaatttag cgtggtatca gcagaaacag
- 181 ggaaaatete etcageteet aatetatget geaacaaaet tageagatgg tgtgeeatea
- 241 aggttcagtg gcagtggatc aggcacacag ttttccctca ggatcaacag cctgcagtct
- 301 gaagattttg ggaggtatta ctgtcaacat ttttggggga ctccgtacac gttcggaggg
- 361 gggaccaaac tggaaataaa ac
- $_{25}$ (13) 1F3 Heavy Chain Variable Region (SEQ ID NO. $_{61)}$
 - 1 <u>atgaactttg ggeteagatt gatttteett gteettgttt</u> <u>taaaaggtgt gaagtgtg</u>ag
 - 61 gtgcagctgg tggagtctgg gggaggctta gtgcagtctg gagggtccct gaaactctcc
 - 121 tgtgcggcct ctggattcac tttcagtaac tatttcatgt cttgggttcg ccagactcca
 - 181 gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactatcca
 - 241 gacagtgtga agggtcgatt caccatctct agagacaatg ccaagaacac cctgtacctg
 - 301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggggat
 - 361 ggttactacg gggactatgc tatggactac tggggtcaag gaaceteagt caeegtetee
 - 421 tcag
- 45 (14) 1F3 Kappa Light Chain Variable Region (SEQ ID NO. 63)
 - 1 <u>atgagtgtgc ccactcaggt cctggggttg ctgctgctgt</u> ggcttacaga tgccagatgt
 - 61 gacatecaga tgaeteagte tecageetee etatetgtat etgtgggaga aaetgteace
 - 121 atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag
 - 181 ggaaaaatete etcageteet ggtetatgat geaacaeaet taecagatgg tgtgecatea
 - 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
 - 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gtttggaggg
 - 361 gggaccagac tggaaattaa ac
 - (15) 3Al2 Heavy Chain Variable Region (SEQ ID NO. 71) $\,$
- 1
 atgaactttg
 ggctcagatt
 gattttcctt
 gtccttgttt

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 taaaaggtgt
 gaagtgtgaa

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61	gtgcagctgg gagggtccct	tggagtctgg gaaaatctcc	gggaggctta	gtgcagcctg

- 121 tgtgcageet etggatttae ttteagtaae tattteatgt ettgggtteg ecagaeteea
- 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
- 241 gacagtgtga agggtcgatt caccatetee agagacaatg ccaagaacae cetgtacetg
- 301 caaatgaaca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggagat
- 361 ggttactatg gggactatgc tatggactac tggggtcaag gaaceteagt caeegtetee
- 421 tcag
- (16) 3A12 Kappa Light Chain Variable Region (SEQ ID NO. 73)
- 1 <u>atgaqtqtqc ccactcaqqt cctqqqqttq ctqctqctqt</u> <u>qqcttacaqa tqccaqatqt</u>
- 61 gacatecaga tgacteagte gecageetee etatetgtat ctgtgggaga aactgteace
- 121 atcacatgtc gagcaagtga gaatatttac attaatttag catggtatca gcagaaacag
- 181 ggaaaaatete etcageteet ggteeatget geaacaaagt tageagatgg tgtgeeatea
- 241 aggttcagtg gcagtggatc aggcacacag tattccctca agatcaacag cctgcagtct
- 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccqtacac qttcqqaqqq
- 361 gggaccaaac tagaaataaa ac

(17) Reference Mouse IgG1 Heavy Chain Constant Region (J00453) (SEQ ID NO. 81)

- 1 ccaaaacgac acceccatct gtctatecac tggecectgg atctgetgec caaactaact
- 61 ccatggtgac cctgggatgc ctggtcaagg gctatttccc tgagccagtg acagtgacct
- 121 ggaactetgg atceetgtee ageggtgtge acacetteee agetgteetg gagtetgace
- 181 totacactot gageagetea gtgactgtee ecteeageee teggeeeage gagaeegtea
- 241 cctgcaacgt tgcccacccg gccagcagca ccaaggtgga caagaaaatt gtgcccaggg
- 301 attgtggttg taagcettge atatgtacag teecagaagt ateatetgte tteatettee
- 361 ccccaaagcc caaggatgtg ctcaccatta ctctgactcc taaggtcacg tgtgttgtgg
- 421 tagacatcag caaggatgat cccgaggtcc agttcagctg gtttgtagat gatgtggagg
- 481 tgcacacagc tcagacgcaa ccccgggagg agcagttcaa cagcactttc cgctcagtca
- 541 gtgaacttee cateatgeae eaggaetgge teaatggeaa ggagtteaaa tgeagggtea
- 601 acagtgcagc tttccctgcc cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
- 661 aggetecaca ggtgtacace attecacete ceaaggagea gatggeeaag gataaagtea

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- 721 gtctgacctg catgataaca gacttcttcc ctgaagacat tactgtggag tggcagtgga
- 781 atgggcagcc agcggagaac tacaagaaca ctcagcccat catgaacacg aatggctctt
- 841 acttegteta cageaagete aatgtgeaga agageaaetg ggaggeagga aataetttea
- 901 cctgctctgt gttacatgag ggcctgcaca accaccatac tgagaagagc ctctcccact
- 961 ctcctggtaa atga
- 15 (18) Mouse IgG1 Heavy Chain Constant Region Determined for 1A3, 1D3, 1F3, and 2B8 (derived from AJ strain mice) (SEQ ID NO. 82)
 - 1 ccaaaacgac acccccatct gtctatccac tggcccctgg atctgctgcc caaactaact
 - 61 ccatggtgac cctgggatgc ctggtcaagg gctatttccc tgagccagtg acagtgacct
 - 121 ggaactetgg atceetgtee ageggtgtge acacetteee agetgteetg cagtetgaee
- 25 181 tetacaetet gageagetea gtgaetgtee eeteegeae etggeeeage gagaeegtea
 - 241 cctgcaacgt tgcccaccg gccagcagca ccaaggtgga caagaaaatt gtgcccaggg
 - 301 attgtggttg taagcettge atatgtacag teecagaagt ateatetgte tteatettee
 - 361 ccccaaagcc caaggatgtg ctcaccatta ctctgactcc taaggtcacg tgtgttgtgg
- 35 421 tagacatcag caaggatgat cccgaggtcc agttcagctg gtttgtagat gatgtggagg
 - 481 tgcacacage teagaegeaa eeeegggagg ageagtteaa cageaettte egeteagtea
- 40 541 gtgaacttee cateatgeae caggaetgge teaatggeaa ggagtteaaa tgeagggtea
 - 601 acagtgcagc tttccctgcc cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
 - 661 aggetecaca ggtgtacace attecacete ecaaggagea gatggeeaag gataaagtea
 - 721 gtctgacctg catgataaca gacttettee etgaagacat taetgtggag tggcagtgga
- 50 781 atgggcagcc agcggagaac tacaagaaca ctcagcccat catggacaca gatggctctt
 - 841 acttcgtcta cagcaagctc aatgtgcaga agagcaactg ggaggcagga aatactttca
- 55 901 cctgctctgt gttacatgag ggcctgcaca accaccatac tgagaagagc ctctcccact
 - 961 ctcctggtaa atga

(19) Reference Mouse Kappa Light Chain ConstantRegion (V00807) and Mouse Kappa Light Chain Constant Region Determined for 1D3, 1F3, and 2B8

- (derived from AJ strain mice) (SEQ ID NO. 83)
 1 gggctgatgc tgcaccaact gtatccatct tcccaccatc
 cagtgagcag ttaacatctg
- 61 gaggtgcete agtegtgtge ttettgaaca aettetaeee caaagacate aatgteaagt

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121	ggaagattga cagttggact	tggcagtgaa gatcaggaca	cgacaaaatg	gcgtcctgaa
181	gcaaagacag gaccaaggac	cacctacagc gagtatgaac	atgagcagca	ccctcacgtt
241	gacataacag aacttcaccc	ctatacctgt attgtcaaga	gaggccactc	acaagacatc
301	gcttcaacag	gaatgagtgt	tag	
(20) term: compa	Mouse Kappa ined for 1A3 ared to 1D3, 34)	a Light Chai 3 containing , 1F3. and 2	in Constant 9 one altere 288 (underli	Region De- ed nucleotide ined) (SEQ ID
1	gggctgatgc cagtgagcag	tgcaccaact ttaacatctg	gtatccatct	tcccaccatc
61	gaggtgeete caaagacate	agtcgtgtgc aatgtcaagt	ttcttgaaca	acttctaccc
121	ggaagattga cagttggact	tggcagtgaa gatcaggaca	cgacaaaatg	gcgtcctgaa
181	gcaaagacag gaccaaggac	cacctacagc gagtatgaac	atgagcagca	ccctca <u>t</u> gtt
241	gacataacag aacttcaccc	ctatacctgt attgtcaaga	gaggccactc	acaagacatc
301	gcttcaacag	gaatgagtgt	tag	

Each of the amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 1 are set forth in FIG. **2**. Each of the sequences are aligned with one another and the sequences defining the signal peptide, CDR_1 , CDR_2 and CDR_3 are identified by boxes. FIG. **3** shows an alignment of the separate 35 CDR_1 , CDR_2 and CDR_3 sequences for each of the antibodies.

Each of the amino acid sequences defining the immunoglobulin light chain variable regions for each of the antibodies produced in Example 1 are set forth in FIG. **4**. Each of the sequences are aligned with one another and the sequences 40 defining the signal peptide, CDR_1 , CDR_2 and CDR_3 are identified by boxes. FIG. **5** shows an alignment of the separate CDR_1 , CDR_2 and CDR_3 sequences for each of the antibodies.

For convenience, Table 1 provides a concordance chart showing the correspondence between the antibody sequences ⁴⁵ discussed in this Example with those presented in the Sequence Listing.

TABLE 1

SEQ. ID NO.	Protein or Nucleic Acid
1	Heavy Chain Variable Region 1A3 - nucleic acid
2	Heavy Chain Variable Region 1A3 - protein
3	Light (kappa) Chain Variable Region 1A3 - nucleic acid
4	Light (kappa) Chain Variable Region 1A3 - protein
5	Heavy Chain CDR ₁ 1A3
6	Heavy Chain CDR ₂ 1A3
7	Heavy Chain CDR ₃ 1A3
8	Light (kappa) Chain CDR ₁ 1A3
9	Light (kappa) Chain CDR ₂ 1A3
10	Light (kappa) Chain CDR ₃ 1A3
11	Heavy Chain Variable Region 2B8 - nucleic acid
12	Heavy Chain Variable Region 2B8 - protein
13	Light (kappa) Chain Variable Region 2B8 - nucleic acid
14	Light (kappa) Chain Variable Region 2B8 - protein
15	Heavy Chain CDR ₁ 2B8
16	Heavy Chain CDR ₂ 2B8
17	Heavy Chain CDR ₃ 2B8
18	Light (kappa) Chain CDR ₁ 2B8

TABLE 1-continued

SEQ. ID NO.	Protein or Nucleic Acid
19	Light (kappa) Chain CDR ₂ 2B8
20	Light (kappa) Chain CDR ₂ 2B8
21	Heavy Chain Variable Region 2F8 - nucleic acid
22	Heavy Chain Variable Region 2F8 - protein
23	Light (kappa) Chain Variable Region 2F8 - nucleic acid
24	Light (happa) Chain Variable Region 2F8 - protein
25	Heavy Chain CDR, 2F8
26	Heavy Chain CDR ₂ 2F8
27	Heavy Chain CDR, 2F8
28	Light (kappa) Chain CDR, 2F8
29	Light (kappa) Chain CDR ₂ 2F8
30	Light (kappa) Chain CDR ₂ 2F8
31	Heavy Chain Variable Region 3B6 - nucleic acid
32	Heavy Chain Variable Region 3B6 - protein
33	Light (kappa) Chain Variable Region 3B6 - nucleic acid
34	Light (kappa) Chain Variable Region 3B6 - protein
35	Heavy Chain CDR, 3B6
36	Heavy Chain CDR ₂ 3B6
37	Heavy Chain CDR ₃ 3B6
38	Light (kappa) Chain CDR, 3B6
39	Light (kappa) Chain CDR ₂ 3B6
40	Light (kappa) Chain CDR ₃ 3B6
41	Heavy Chain Variable Region 3D11 - nucleic acid
42	Heavy Chain Variable Region 3D11 - protein
43	Light (kappa) Chain Variable Region 3D11 - nucleic acid
44	Light (kappa) Chain Variable Region 3D11 - protein
45	Heavy Chain CDR ₁ 3D11
46	Heavy Chain CDR ₂ 3D11
47	Heavy Chain CDR ₃ 3D11
48	Light (kappa) Chain CDR ₁ 3D11
49	Light (kappa) Chain CDR ₂ 3D11
50	Light (kappa) Chain CDR ₃ 3D11
51	Heavy Chain Variable Region 1D3 - nucleic acid
52	Heavy Chain Variable Region 1D3 - protein
53	Light (kappa) Chain Variable Region 1D3 - nucleic acid
54	Light (kappa) Chain Variable Region 1D3 - protein
55	Heavy Chain CDR ₁ 1D3
56	Heavy Chain CDR ₂ 1D3
57	Heavy Chain CDR ₃ 1D3
58	Light (kappa) Chain CDR ₁ 1D3
59	Light (kappa) Chain CDR ₂ 1D3
60	Light (kappa) Chain CDR ₃ 1D3
61	Heavy Chain Variable Region 1F3 - nucleic acid
62	Heavy Chain Variable Region 1F3 - protein
63	Light (kappa) Chain Variable Region 1F3 - nucleic acid
64	Light (kappa) Chain Variable Region 1F3 - protein
65	Heavy Chain CDR ₁ 1F3
66	Heavy Chain CDR ₂ 1F3
67	Heavy Chain CDR ₃ 1F3
68	Light (kappa) Chain CDR ₁ 1F3
69	Light (kappa) Chain CDR ₂ 1F3
70	Light (kappa) Chain CDR ₃ 1F3
71	Heavy Chain Variable Region 3A12 - nucleic acid
72	Heavy Chain Variable Region 3A12 - protein
73	Light (kappa) Chain Variable Region 3A12 - nucleic acid
74	Light (kappa) Chain Variable Region 3A12 - protein
75	Heavy Chain CDR ₁ $3A12$
76	Heavy Chain CDR ₂ 3A12
//	Heavy Chain CDR ₃ 3A12
78	Light (kappa) Chain CDR ₁ 3A12
79	Light (kappa) Chain $CDR_2 3A12$
80	Light (kappa) Chain CDR ₃ 3A12

Also, for convenience, the following sequences represent the actual or contemplated full length heavy and light chain sequences (i.e., containing both the variable and constant region sequences) for each of the antibodies described in this Example. It is noted that the constant regions of the murine antibodies 2F8, 3A12, 3B6, and 3D11 were not sequenced but are presumed to have the same constant region sequences as the 1D3, 1F3, and 2B8 antibodies, which were sequenced, as they were all derived from AJ strain mice. It is appreciated, however, that the variable region sequences described herein can be ligated to each of a number of other constant region

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sequences known to those skilled in the art to produce active full length immunoglobulin heavy and light chains.

(1) Nucleic Acid Sequence Encoding the Full Length
 1A3 Heavy Chain Sequence (1A3 Heavy Chain Variable
 Region and IgG1 Constant Region) (signal sequence
 underlined) (SEO ID NO. 122)

- 1 <u>atqaactttq qqctcaqatt qattttcctt qtccttqttt</u> <u>taaaaqgtqt gaagtqtg</u>aa
- 61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
- 121 tgtgcagcct ctgaattcac tttcagtaac tattacatgt cttgggttcg ccagactcca
- 181 gagaagaggc tgcagtgggt cgcatacatt agtcctggtg gtggtagctc ctactatcca
- 241 gccagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
- 301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgcaag acaaggggat
- 361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
- 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
- 481 aactecatgg tgaceetggg atgeetggte aagggetatt teeetgagee agtgaeagtg
- 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
- 601 gacetetaca etetgageag eteagtgaet gteceeteea geacetggee eagegagaee
- 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
- 721 agggattgtg gttgtaagee ttgeatatgt acagteeeag aagtateate tgtetteate
- 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
- 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
- 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
- 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
- 1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctccaaaac caaaggcaga
- 1081 ccgaaggctc cacaggtgta caccattcca cctcccaagg agcagatggc caaggataaa
- 1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
- 1201 tggaatgggc agccagcgga gaactacaag aacactcagc ccatcatgga cacagatggc
- 1261 tettaetteg tetacageaa geteaatgtg eagaagagea actgggagge aggaaataet
- 1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
- 1381 cacteteetg gtaaatga

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(2) Protein Sequence Defining the Full Length 1A3 Heavy Chain Sequence (1A3 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 123)

- 1 evqlvesggg lvqpggslkl scaaseftfs nyymswvrqt pekrlqwvay ispgggssyy
- 61 pasvkgrfti srdnakntly lqmsslksed tamyycarqg dgyygdyamd ywgqgtsvtv
- 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
- 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
- 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
- 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
- 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
- 421 tftcsvlheg lhnhhteksl shspgk
- ²⁵ (3) Nucleic Acid Sequence Encoding the Full Length 1A3 Light Chain Sequence (1A3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEO ID NO. 124)
 - 1 <u>atgagtgtgc ccactcaggt cctggggttg ctgctgctgt</u> <u>ggcttacaga tgccagatgt</u>
 - 61 gacatecaga tgactcagte tecageetee etatetgttt etgtgggaga aactgteace
 - 121 atcacatgtc gagcaagtga gaatatttat agtaatttag catggtatca gcagaaacag
 - 181 ggaaaatete etcageteet ggtetatget geaacaaaet tageagatgg tgtgeeatea
 - 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
 - 301 gaagattttg ggacttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
 - 361 gggaccaagc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 - 421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 - 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 - 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcatg
 - 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 - 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

(4) Protein Sequence Defining the Full Length 1A360 Light Chain Sequence (1A3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 125)

- 1 diqmtqspas lsvsvgetvt itcraseniy snlawyqqkq gkspqllvya atnladgvps
- 61 rfsgsgsgtq fslkinslqs edfgtyycqh fwgtpytfgg gtkleikrad aaptvsifpp

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- 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlm
- 181 ltkdeyerhn sytceathkt stspivksfn rnec

(5) Nucleic Acid Sequence Encoding the Full Length 2B8 Heavy Chain Sequence (2B8 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 126)

- 1 <u>atgggatgga gctatatcat cctctttttg gtagcaacag</u> <u>ctacagatgt ccactcc</u>cag
- 61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
- 121 tgcaaggett etggetacae etteaceaee taetggatge aetgggtgaa teagaggeet
- 181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
- 241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccaqcac aqcctacatq
- 301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
- 361 ggtagcatet ttgaetaetg gggeeaagge accaetetea cagteteete ageeaaaaeg
- 421 acacceccat ctgtctatec actggeceet ggatetgetg cccaaactaa ctecatggtg
- 481 accctgggat gcctggtcaa gggctatttc cctgagccag tgacagtgac ctggaactct
- 541 ggatecetgt ceageggtgt geacacette ceagetgtee tgeagtetga cetetaeaet
- 601 ctgagcaget cagtgactgt cecetecage acetggeeca gegagaeegt cacetgeaae
- 661 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt
- 721 tgtaagcett geatatgtae agteeeagaa gtateatetg tetteatett eeeceaaag
- 781 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc
- 841 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca
- 901 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt
- 961 cccatcatgc accaggactg gctcaatggc aaggagttca aatgcagggt caacagtgca
- 1021 gctttccctg cccccatcga gaaaaccatc tccaaaacca aaggcagacc gaaggctcca
- 1081 caggtgtaca ccattccacc tcccaaggag cagatggcca aggataaagt cagtctgacc
- 1141 tgcatgataa cagacttett eeetgaagae attactgtgg agtggcagtg gaatgggcag
- 1201 ccageggaga actacaagaa cacteageee ateatggaca cagatggete ttacttegte
- 1261 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct
- 1321 gtgttacatg agggeetgea caaceaceat aetgagaaga geeteteeea eteteetggt
- 1381 aaatga

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(6) Protein Sequence Defining the Full Length 2B8 Heavy Chain Sequence (2B8 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 127)

- 1 qvqlqqpgae lvkpgtsvkl sckasgytft tywmhwvnqr pgqglewige inptnghtny
- 61 nekfkskatl tvdkssstay mqlssltsed savyycarny vgsifdywgq gttltvssak
- 121 ttppsvypla pgsaaqtnsm vtlgclvkgy fpepvtvtwn sgslssgvht fpavlqsdly
- 181 tlsssvtvps stwpsetvtc nvahpasstk vdkkivprdc gckpcictvp evssvfifpp
- 241 kpkdvltitl tpkvtcvvvd iskddpevqf swfvddvevh taqtqpreeq fnstfrsvse
- 301 lpimhqdwln gkefkcrvns aafpapiekt isktkgrpka pqvytipppk eqmakdkvsl
- 361 tcmitdffpe ditvewqwng qpaenykntq pimdtdgsyf vysklnvqks nweagntftc
- 421 svlheglhnh htekslshsp gk
- ²⁵ (7) Nucleic Acid Sequence Encoding the Full Length 2B8 Light Chain Sequence (2B8 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEO ID NO. 128)
 - 1 <u>atggaatcac agactetggt etteatatee atactgetet</u> <u>ggttatatgg tgetgatggg</u>
 - 61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
 - 121 ttgagetgea aggeeagtga gaatgtggtt tettatgtat cetggtatea acagaaacea
 - 181 gcgcagtete etaaactget gatataeggg geateeaace ggaacaetgg ggteeeegat
 - 241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
 - 301 gaagacettg cagattatea etgtgggeag agttacaaet ateeqtacae gtteggaggg
 - 361 gggaccaggc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 - 421 tecagtgage agttaacate tggaggtgee teagtegtgt gettettgaa caacttetae
 - 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 - 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 - 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 - 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

(8) Protein Sequence Defining the Full Length 2B860 Light Chain Sequence (2B8 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 129)

- 1 nivmtqspks msmsvgervt lsckasenvv syvswyqqkp aqspklliyg asnrntgvpd
- 61 rftgsgsatd ftltissvra edladyhcgq synypytfgg gtrleikrad aaptvsifpp

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- 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
- 181 ltkdeyerhn sytceathkt stspivksfn rnec

(9) Nucleic Acid Sequence Encoding the Full Length 2F8 Heavy Chain Sequence (2F8 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 130)

- 1 <u>atggaatgga getgggtett tetetteete etgteagtaa</u> <u>etgeaggtgt ceaetge</u>eag
- 61 gtccagctga agcagtctgg agctgagctg gtgaggcctg ggacttcagt gaagatgtcc
- 121 tgcaaggett etggetacae etteactaee taetatatae aetgggtgaa teagaggeet
- 181 ggacagggcc ttgagtggat tggaaagatt ggtcctggaa gtggtagtac ttactacaat
- 241 gagatgttca aagacaaggc cacattgact gtagacacat cctccagcac agcctacatg
- 301 cageteagea geetgacate tgaegaetet geggtetatt tetgtgeaag aaggggaetg
- 361 ggacgtggct ttgactactg gggccaaggc accactctca cagtctcctc agccaaaacg
- 421 acacceccat ctgtctatec actggeceet ggatetgetg cccaaactaa ctecatggtg
- 481 accctgggat gcctggtcaa gggctatttc cctgagccag tgacagtgac ctggaactct
- 541 ggatecetgt ceageggtgt geacacette ceagetgtee tgeagtetga cetetaeaet
- 601 ctgagcaget cagtgactgt cecetecage acetggeeca gegagaeegt cacetgeaae
- 661 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt
- 721 tgtaagcett geatatgtae agteeeagaa gtateatetg tetteatett eeeceaaag
- 781 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc
- 841 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca
- 901 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt
- 961 cccatcatgc accaggactg gctcaatggc aaggagttca aatgcagggt caacagtgca
- 1021 gctttccctg cccccatcga gaaaaccatc tccaaaacca aaggcagacc gaaggctcca
- 1081 caggtgtaca ccattccacc tcccaaggag cagatggcca aggataaagt cagtctgacc
- 1141 tgcatgataa cagacttett eeetgaagae attactgtgg agtggeagtg gaatgggeag
- 1201 ccageggaga actacaagaa cacteageee ateatggaca cagatggete ttacttegte
- 1261 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct
- 1321 gtgttacatg agggeetgea caaceaceat aetgagaaga geeteteeea eteteetggt
- 1381 aaatga

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(10) Protein Sequence Defining the Full Length 2F8 Heavy Chain Sequence (2F8 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 131)

- 1 qvqlkqsgae lvrpgtsvkm sckasgytft tyyihwvngr pgqglewigk igpgsgstyy
- 61 nemfkdkatl tvdtssstay mqlssltsdd savyfcarrg lgrgfdywgq gttltvssak
- 121 ttppsvypla pgsaaqtnsm vtlgclvkgy fpepvtvtwn sgslssgvht fpavlqsdly
- 181 tlsssvtvps stwpsetvtc nvahpasstk vdkkivprdc gckpcictvp evssvfifpp
- 241 kpkdvltitl tpkvtcvvvd iskddpevqf swfvddvevh taqtqpreeq fnstfrsvse
- 301 lpimhqdwln gkefkcrvns aafpapiekt isktkgrpka pqvytipppk eqmakdkvsl
- 361 tcmitdffpe ditvewqwng qpaenykntq pimdtdgsyf vysklnvqks nweagntftc
- 421 svlheglhnh htekslshsp gk
- ²⁵ (11) Nucleic Acid Sequence Encoding the Full Length 2F8 Light Chain Sequence (2F8 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 132)
 - 1 <u>atggagacag acacaatcet getatgggtg etgetgetet</u> <u>gggttecagg etceaetggt</u>
 - 61 gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc
 - 121 atctcctgca aggccagcca aagtgttgat tatgatggta atagttatat caactggtac
 - 181 caacagaaac caggacagcc acccaaagtc ctcatctatg ttgcatccaa tctagaatct
 - 241 gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat
 - 301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc aaagtattga ggatcctccc
 - 361 acgttcggtg ctggggaccaa gctggagctg aaacgggctg atgctgcacc aactgtatcc
 - 421 atcttcccac catccagtga gcagttaaca tctggaggtg cctcagtcgt gtgcttcttg
 - 481 aacaacttct accccaaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa
 - 541 aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc
 - 601 agcaccetea egttgaceaa ggaegagtat gaaegaeata acagetatae etgtgaegee
 - 661 actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgttag

(12) Protein Sequence Defining the Full Length 2F860 Light Chain Sequence (2F8 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 133)

- 1 divltqspas lavslgqrat isckasqsvd ydgnsyinwy qqkpgqppkv liyvasnles
- 61 giparfsgsg sgtdftlnih pveeedaaty ycqqsiedpp tfgagtklel kradaaptvs

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- 121 ifppsseqlt sggasvvcfl nnfypkdinv kwkidgserq ngvlnswtdq dskdstysms
- 181 stltltkdey erhnsytcea thktstspiv ksfnrnec

(13) Nucleic Acid Sequence Encoding the Full Length 3B6 Heavy Chain Sequence (3B6 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 134)

- 1 <u>atggaatggc cttgtatett tetetteete etgteagtaa</u> <u>ctgaaggtgt ceaetee</u>ag
- 61 gttcagctgc agcagtctgg ggctgaactg gtgaggcctg qqtcctcagt gaagatttcc
- 121 tgcaaggett etggetatgt atteagtage taetggatga aetgggtgaa geagaggeet
- 181 ggacagggtc ttgagtggat tggacagatt tatcctggag atggtgatag taactacaat
- 241 ggaaacttca agggtaaagc cacactgact gcagacaaat cetecaqtac agectacatg
- 301 cageteagea geetaacate tgaggaetet geggtetatt tetgtgeate ceageteggg
- 361 ctacgtgaga actactttga ctactggggc caaggcacca cteteacagt eteeteagee
- 421 aaaacgacac ccccatctgt ctatccactg gcccctggat ctgctgccca aactaactcc
- 481 atggtgaccc tgggatgcct ggtcaagggc tatttccctg aqccaqtqac aqtqacctqq
- 541 aactetggat eeetgteeag eggtgtgeae acetteeeag etgteetgea gtetgaeete
- 601 tacactetga geageteagt gaetgteece teeageaeet ggeeeagega gaeegteaee
- 661 tgcaacgttg cccacccggc cagcagcacc aaggtggaca agaaaattgt gcccagggat
- 721 tgtggttgta agcettgeat atgtacagte ecagaagtat catetgeett catetteece
- 781 ccaaagccca aggatgtgct caccattact ctgactccta aggtcacgtg tgttgtggta
- 841 gacatcagca aggatgatcc cgaggtccag ttcagctggt ttgtagatga tgtggaggtg
- 901 cacacagete agaegeaace eegggaggag cagtteaaca geaetteeg eteagteagt
- 961 gaactteeca teatgeacea ggaetggete aatggeaagg agtteaaatg eagggteaae
- 1021 agtgcagett teeetgeeee categagaaa aceateteea aaaceaaagg cagacegaag
- 1081 gctccacagg tgtacaccat tccacctccc aaggagcaga tggccaagga taaagtcagt
- 1141 ctgacctgca tgataacaga cttcttccct gaagacatta ctgtggagtg gcagtggaat
- 1201 gggcagccag cggagaacta caagaacact cagcccatca tggacacaga tggctcttac
- 1261 ttcgtctaca gcaagctcaa tgtgcagaag agcaactggg aggcaggaaa tactttcacc
- 1321 tgctctgtgt tacatgaggg cctgcacaac caccatactg agaagagcct ctcccactct
- 1381 cctggtaaat ga

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(14) Protein Sequence Defining the Full Length 3B6 Heavy Chain Sequence (3B6 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 135)

- 1 qvqlqqsgae lvrpgssvki sckasgyvfs sywmnwvkqr pgqglewigq iypgdgdsny
- 61 ngnfkgkatl tadkssstay mqlssltsed savyfcasql glrenyfdyw gqgttltvss
- 121 akttppsvyp lapgsaaqtn smvtlgclvk gyfpepvtvt wnsgslssgv htfpavlqsd
- 181 lytlsssvtv psstwpsetv tcnvahpass tkvdkkivpr dcgckpcict vpevssvfif
- 241 ppkpkdvlti tltpkvtcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
- 301 selpimhqdw lngkefkcrv nsaafpapie ktisktkgrp kapqvytipp pkeqmakdkv
- 361 sltcmitdff peditvewqw ngqpaenykn tqpimdtdgs yfvysklnvq ksnweagntf
- 421 tcsvlheglh nhhtekslsh spgk
- ²⁵ (15) Nucleic Acid Sequence Encoding the Full Length 3B6 Light Chain Sequence (3B6 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 136)
 - 1 <u>ATGgacATGa ggacccctgc tcagtttctt ggaatcttgt</u> <u>tgctctggtt tccaggtatc</u>
 - 61 <u>aaatgtg</u>aca tcaagatgac ccagtctcca tcttccatgt atgcatctct aggagagaga
 - 121 gtcacaatca cttgcaaggc gagtcaggac attaaaagct atttaagctg gttccagcag
 - 181 aaaccaggga aatctcctaa gaccctgatc tatcgtgtaa acagattggt agatggggtc
 - 241 ccatcaaggt tcagtggcag tggatctggg caagattett ctetcaccat caccageetg
 - 301 gagaatgaag atatgggaat ttattattgt ctacagtatg atgagtttcc gttcacgttc
 - 361 ggagggggga ccaagetgga aataaagegg getgatgetg caecaaetgt atceatette
 - 421 ccaccatcca gtgagcagtt aacatctgga ggtgcctcag tcgtgtgctt cttgaacaac
 - 481 ttctacccca aagacatcaa tgtcaagtgg aagattgatg gcagtgaacg acaaaatggc
 - 541 gtcctgaaca gttggactga tcaggacagc aaagacagca cctacagcat gagcagcacc
 - 601 ctcacgttga ccaaggacga gtatgaacga cataacagct atacctgtga qqccactcac
 - 661 aagacatcaa cttcacccat tgtcaagagc ttcaacagga atgagtgtta g

(16) Protein Sequence Defining the Full Length 3B660 Light Chain Sequence (3B6 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 137)

- 1 dikmtqspss myaslgervt itckasqdik sylswfqqkp gkspktliyr vnrlvdgvps
- 61 rfsgsgsggd ssltitslen edmgiyyclq ydefpftfgg gtkleikrad aaptvsifpp

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- 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
- 181 ltkdeyerhn sytceathkt stspivksfn rnec

(17) Nucleic Acid Sequence Encoding the Full Length 3D11 Heavy Chain Sequence (3D11 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 138)

- 1 <u>atggetgtec eggtgetgtt eetetgeetg gttgeattte</u> <u>caagetgtgt eetgtee</u>cag
- 61 gtacagetga aggagteagg acetggeetg gtggegeeet caeaqageet gteeateact
- 121 tgcactgtct ctgggttttc attaaccagc tatagtttac actgggttcg ccagceteca
- 181 ggaaagggtc tggaatggct gggagtaata tgggctggtg gaaacacaaa ttataattcg
- 241 teteteatgt ecagaetgae cateaggaaa gaeaaeteea agageeaagt tttettaaaa
- 301 atgaacagtc tgcaaactga tgacacagcc atgtactact gtgccagaga gaggtttgct
- 361 tactggggcc aagggactct ggtcactgtc tctgcagcca aaacgacacc cccatctgtc
- 421 tatccactgg cccctggatc tgctgcccaa actaactcca tggtgaccct gggatgcctg
- 481 gtcaagggct atttccctga gccagtgaca gtgacctgga actctggatc cctgtccagc
- 541 ggtgtgcaca ccttcccagc tgtcctgcag tctgacctct acactctgag cagctcagtg
- 601 actgtcccct ccagcacctg gcccagcgag accgtcacct gcaacgttgc ccacccggcc
- 661 agcagcacca aggtggacaa gaaaattgtg cccagggatt gtggttgtaa gccttgcata
- 721 tgtacagtec cagaagtate atetgtette atetteecee caaageecaa ggatgtgete
- 781 accattactc tgactcctaa ggtcacgtgt gttgtggtag acatcaqcaa qqatqatccc
- 841 gaggtccagt tcagctggtt tgtagatgat gtggaggtgc acacagetca gacgcaaccc
- 901 cgggaggagc agttcaacag cactttccgc tcagtcagtg aacttcccat catgcaccag
- 961 gactggetca atggcaagga gttcaaatge agggtcaaca gtgcagettt ceetgeeeee
- 1021 atcgagaaaa ccatctccaa aaccaaaggc agaccgaagg ctccacaggt gtacaccatt
- 1081 ccacctccca aggagcagat ggccaaggat aaagtcagtc tgacctgcat gataacagac
- 1141 ttcttccctg aagacattac tgtggagtgg cagtggaatg ggcagccagc ggagaactac
- 1201 aagaacactc agcccatcat ggacacagat ggctcttact tcgtctacag caagctcaat
- 1261 gtgcagaaga gcaactggga ggcaggaaat actttcacct gctctgtgtt acatgagggc
- 1321 ctgcacaacc accatactga gaagageete teceactete ctggtaaatg a

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- (18) Protein Sequence Defining the Full Length
- 3D11 Heavy Chain Sequence (3D11 Heavy Chain Varia-5 ble Region and IgG1 Constant Region) (without sig
 - nal sequence) (SEQ ID NO. 139) 1 qvqlkesgpg lvapsqslsi tctvsgfslt syslhwvrqp
 - i qvqikesgpg ivapsqsisi tetvsgisit sysinwvrqp pgkglewlgv iwaggntnyn
 - 61 sslmsrltir kdnsksqvfl kmnslqtddt amyycarerf aywgqgtlvt vsaakttpps
 - 121 vyplapgsaa qtnsmvtlgc lvkgyfpepv tvtwnsgsls sgvhtfpavl qsdlytlsss
 - 181 vtvpsstwps etvtcnvahp asstkvdkki vprdcgckpc ictvpevssv fifppkpkdv
 - 241 ltitltpkvt cvvvdiskdd pevqfswfvd dvevhtaqtq preeqfnstf rsvselpimh
 - 301 qdwlngkefk crvnsaafpa piektisktk grpkapqvyt ipppkeqmak dkvsltcmit
 - 361 dffpeditve wqwngqpaen ykntqpimdt dgsyfvyskl nvqksnweag ntftcsvlhe
 - 421 glhnhhteks lshspgk
- ²⁵ (19) Nucleic Acid Sequence Encoding the Full Length 3D11 Light Chain Sequence (3D11 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 140)
 - 1 <u>atggattttc aagtgcagat tttcagcttc ctgctaatca</u> <u>gtgcctcagt caaaatatcc</u>
 - 61 <u>agagga</u>caaa ttgttctcac ccagtctcca gcaatcatgt ctgcatatcc aggggagaag
 - 121 gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgcactggta ccagcagaag
 - 181 tcaggcacct cccccaaaag atggatttat gacacatcca aactggcttc tggagtccct
 - 241 getegettea gtggeagtgg gtetgggaee tettaeteee teacaateag tagtatggag
 - 301 gctgaagatg ctgccactta ttactgccag cagtggagta gtaacccact cacgttcggt
 - 361 gctgggacca agctggagct gaaacgggct gatgctgcac caactgtatc catcttccca
 - 421 ccatccagtg agcagttaac atctggaggt gcctcagtcg tgtgcttctt gaacaacttc
 - 481 taccccaaag acatcaatgt caagtggaag attgatggca gtgaacgaca aaatggcgtc
 - 541 ctgaacagtt ggactgatca ggacagcaaa gacagcacct acagcatgag cagcaccctc
 - 601 acgttgacca aggacgagta tgaacgacat aacagctata cctgtgaggc cactcacaag
 - 661 acatcaactt cacccattgt caagagette aacaggaatg agtgttag
 - (20) Protein Sequence Defining the Full Length
- 60 3D11 Light Chain Sequence (3D11 Kappa Variable Region and Constant Region) (without signal se
 - quence) (SEQ ID NO. 141)
 1 qivltqspai msaypgekvt mtcsasssvs ymhwyqqksg
 - tspkrwiydt sklasgvpar
 - 61 fsgsgsgtsy sltissmeae daatyycqqw ssnpltfgag tklelkrada aptvsifpps

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- 121 seqltsggas vvcflnnfyp kdinvkwkid gserqngvln swtdqdskds tysmsstltl
- 181 tkdeyerhns ytceathkts tspivksfnr nec

(21) Nucleic Acid Sequence Encoding the Full Length 1D3 Heavy Chain Sequence (1D3 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 142)

- 1 <u>atgaactttg ggctcagatt gattttcctt gtccttgttt</u> <u>taaaaggtgt gaagtgtg</u>aa
- 61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg
 gagggtccct gaaactctcc
- 121 tgtgcagcct ctggattcac tttcagtgac tattacatgt cttgggttcg ccagactcca
- 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
- 241 gacagtgtga agggtcgatt caccatetee cgagacaatg ccaagaacae cetgtacetg
- 301 caaatgagca gtctgaagtc tgaggacaca gccatatatt actgtgtgag acaaggggat
- 361 ggttattacg gggactatgc tatggactac tggggtcaag gaacctcagt catcgtctcc
- 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
- 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
- 541 acctggaact ctggatccct gtccageggt gtgcacacet tcccagetgt cctgcagtet
- 601 gacetetaca etetgageag eteagtgaet geeeeteea geacetggee eagegagaee
- 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
- 721 agggattgtg gttgtaagee ttgeatatgt acagteeeag aagtateate tgtetteate
- 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
- 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
- 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
- 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
- 1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctcccaaaac caaaggcaga
- 1081 ccgaaggetc cacaggtgta caccatteca ecteecaagg ageagatgge caaggataaa
- 1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
- 1201 tggaatgggc agccagegga gaactacaag aacactcagc ccatcatgga cacagatggc
- 1261 tettaetteg tetaeageaa geteaatgtg eagaagagea actgggagge aggaaataet
- 1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
- 1381 cacteteetg gtaaatga

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 (22) Protein Sequence Defining the Full Length 1D3 Heavy chain sequence (1D3 Heavy Chain Variable Re-5 gion and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 143)

- 1 evqlvesggg lvqpggslkl scaasgftfs dyymswvrqt pekrlewvay issgggstyy
- 61 pdsvkgrfti srdnakntly lqmsslksed taiyycvrqg dgyygdyamd ywgqgtsviv
- 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
- 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
- 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
- 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
- 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsytvyskln vqksnweagn
- 421 tttcsvlheg lhnhhteksl shspgk
- ²⁵ (23) Nucleic Acid Sequence Encoding the Full Length 1D3 Light Chain Sequence (1D3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 144)
 - 1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgtcagatgt
 - 61 gacatacaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc
 - 121 atcacatgtc gaacaagtga gaatatttac agtaatttag cgtggtatca gcagaaacag
 - 181 ggaaaatete etcageteet aatetatget geaacaaaet tageagatgg tgtgeeatea
 - 241 aggttcagtg gcagtggatc aggcacacag ttttccctca ggatcaacag cctgcagtct
 - 301 gaagattttg ggaggtatta ctgtcaacat ttttggggga ctccgtacac gttcggaggg
 - 361 gggaccaaac tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 - 421 tecagtgage agttaacate tggaggtgee teagtegtgt gettettgaa caacttetae
 - 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 - 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 - 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 - 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

(24) Protein Sequence Defining the Full Length 1D360 Light Chain Sequence (1D3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 145)

- 1 diqmtqspas lsvsvgetvt itcrtseniy snlawyqqkq gkspqlliya atnladgvps
- 61 rfsgsgsgtq fslrinslqs edfgryycqh fwgtpytfgg gtkleikrad aaptvsifpp

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- 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
- 181 ltkdeyerhn sytceathkt stspivksfn rnec

(25) Nucleic Acid Sequence Encoding the Full Length 1F3 Heavy Chain Sequence (1F3 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEO ID NO. 146)

- 1 <u>atgaactttg ggctcagatt gattttcctt gtccttgttt</u> <u>taaaaggtgt gaagtgtg</u>ag
- 121 tgtgcggcct ctggattcac tttcagtaac tattcatgt cttgggttcg ccagactcca
- 181 gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactatcca
- 241 gacagtgtga agggtcgatt caccatetet agagacaatg ceaagaacae eetgtaeetg
- 301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggggat
- 361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
- 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
- 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
- 541 acctggaact ctggatccct gtccageggt gtgcacacet tcccagetgt cctgcagtet
- 601 gacetetaca etetgageag eteagtgaet geeeeteea geacetggee eagegagaee
- 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
- 721 agggattgtg gttgtaagee ttgeatatgt acagteeeag aagtateate tgtetteate
- 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
- 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
- 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
- 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
- 1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctcccaaaac caaaggcaga
- 1081 ccgaaggetc cacaggtgta caccatteca ecteecaagg ageagatgge caaggataaa
- 1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
- 1201 tggaatgggc agccagegga gaactacaag aacactcagc ccatcatgga cacagatggc
- 1261 tettaetteg tetaeageaa geteaatgtg eagaagagea actgggagge aggaaataet
- 1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
- 1381 cacteteetg gtaaatga

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- (26) Protein Sequence Defining the Full Length1F3 Heavy Chain Sequence (1F3 Heavy Chain Variable⁵ Region and IgG1 Constant Region) (without signal
 - sequence) (SEQ ID NO. 147)
 1 evqlvesggg lvqsggslkl scaasgftfs nyfmswvrqt
 - pekrlewvay issgggatyy
 - 61 pdsvkgrfti srdnakntly lqmsslksed tamyycvrqg dgyygdyamd ywgqgtsvtv
 - 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
 - 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
 - 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
 - 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
 - 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
 - 421 tftcsvlheg lhnhhteksl shspgk
- ²⁵ (27) Nucleic Acid Sequence Encoding the Full Length 1F3 Light Chain Sequence (1F3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 148)
 - 1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
 - 61 gacatecaga tgaeteagte tecageetee etatetgtat etgtgggaga aaetgteace
 - 121 atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag
 - 181 ggaaaatete etcageteet ggtetatgat geaacaeaet taccagatgg tgtgeeatea
 - 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
 - 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gtttggaggg
 - 361 gggaccagac tggaaattaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 - 421 tecagtgage agttaacate tggaggtgee teagtegtgt gettettgaa caacttetae
 - 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 - 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 - 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 - 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

(28) Protein Sequence Defining the Full Length 1F360 Light Chain Sequence (1F3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 149)

- 1 diqmtqspas lsvsvgetvt itcraseniy snlawyqqkq gkspqllvyd athlpdgvps
- 61 rfsgsgsgtq fslkinslqs edfgsyycqh fwgtpytfgg gtrleikrad aaptvsifpp

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- 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
- 181 ltkdeyerhn sytceathkt stspivksfn rnec

(29) Nucleic Acid Sequence Encoding the Full Length 3A12 Heavy Chain Sequence (3A12 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 150)

- 1 <u>atgaactttg ggeteagatt gatttteett gteettgttt</u> <u>taaaaggtgt gaagtgtg</u>aa
- 61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg qaqqqtccct gaaaatctcc
- 121 tgtgcagcct ctggatttac tttcagtaac tatttcatgt cttgggttcg ccagactcca
- 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
- 241 gacagtgtga agggtcgatt caccatetee agagacaatg ccaagaacae cetgtacetg
- 301 caaatgaaca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggagat
- 361 ggttactatg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
- 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
- 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
- 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
- 601 gacetetaca etetgageag eteagtgaet geeeeteea geacetggee eagegagaee
- 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
- 721 agggattgtg gttgtaagee ttgeatatgt acagteeeag aagtateate tgtetteate
- 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
- 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
- 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
- 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
- 1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctcccaaaac caaaggcaga
- 1081 ccgaaggetc cacaggtgta caccatteca ecteecaagg ageagatgge caaggataaa
- 1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
- 1201 tggaatgggc agccagegga gaactacaag aacactcagc ccatcatgga cacagatggc
- 1261 tettaetteg tetaeageaa geteaatgtg eagaagagea actgggagge aggaaataet
- 1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
- 1381 cacteteetg gtaaatga

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- (30) Protein Sequence Defining the Full Length 3A12 Heavy Chain Sequence (3A12 Heavy Chain Varia-
- 5 ble Region and IgGL Constant Region) (without signal sequence) (SEQ ID NO. 151)
 - 1 evqlvesggg lvqpggslki scaasgftfs nyfmswvrqt pekrlewvay issgggstyy
 - 61 pdsvkgrfti srdnakntly lqmnslksed tamyycvrqg dgyygdyamd ywgqgtsvtv
 - 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
 - 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
 - 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
 - 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
 - 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
 - 421 tftcsvlheg lhnhhteksl shspgk
- ²⁵ (31) Nucleic Acid Sequence Encoding the Full Length 3A12 Light Chain Sequence (3A12 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 152)
 - 1 <u>atgagtgtgc ccactcaggt cctggggttg ctgctgctgt</u> <u>ggcttacaga tgccagatgt</u>
 - 61 gacatecaga tgaeteagte gecageetee etatetgtat etgtgggaga aaetgteace
 - 121 atcacatgtc gagcaagtga gaatatttac attaatttag catggtatca gcagaaacag
 - 181 ggaaaatete etcageteet ggteeatget geaacaaagt tageagatgg tgtgeeatea
 - 241 aggttcagtg gcagtggatc aggcacacag tattccctca agatcaacag cctgcagtct
 - 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
 - 361 gggaccaaac tagaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 - 421 tecagtgage agttaacate tggaggtgee teagtegtgt gettettgaa caacttetae
 - 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 - 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 - 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 - 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

(32) Protein Sequence Defining the Full Length

- 60 3A12 Light Chain Sequence (3A12 Kappa Variable Region and Constant Region) (without signal se
 - quence) (SEQ ID NO. 153)
 1 digmtqspas lsvsvgetvt itcraseniy inlawyqqkq
 - gkspqllvha atkladgvps
 - 61 rfsgsgsgtq yslkinslqs edfgsyycqh fwgtpytfgg gtkleikrad aaptvsifpp

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sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl 121 nswtdqdskd stysmsstlt

181 ltkdeverhn svtceathkt stspivksfn rnec

For convenience, Table 2 provides a concordance chart showing the correspondence between the full length sequences of the antibodies discussed in this Example with those presented in the Sequence Listing.

TABLE 2

SEQ. ID NO.	Protein or Nucleic Acid	15
122	1A3 Heavy Variable + IgG1 constant - nucleic acid	
123	1A3 Heavy Variable + IgG1 constant - protein	
124	1A3 Light Variable + constant - nucleic acid	
125	1A3 Light Variable + constant - protein	20
126	2B8 Heavy Variable + IgG1 constant - nucleic acid	
127	2B8 Heavy Variable + IgG1 constant - protein	
128	2B8 Light Variable + constant - nucleic acid	
129	2B8 Light Variable + constant - protein	
130	2F8 Heavy Variable + IgG1 constant - nucleic acid	
131	2F8 Heavy Variable + IgG1 constant - protein	25
132	2F8 Light Variable + constant - nucleic acid	
133	2F8 Light Variable + constant - protein	
134	3B6 Heavy Variable + IgG1 constant - nucleic acid	
135	3B6 Heavy Variable + IgG1 constant - protein	
136	3B6 Light Variable + constant - nucleic acid	
137	3B6 Light Variable + constant - protein	30
138	3D11 Heavy Variable + IgG1 constant - nucleic acid	
139	3D11 Heavy Variable + IgG1 constant - protein	
140	3D11 Light variable + constant - nucleic acid	
141	3D11 Light Variable + constant - protein	
142	1D3 Heavy variable + IgG1 constant - nucleic acid	
143	1D3 Heavy variable + IgG1 constant - protein	35
144	1D3 Light Variable + constant - nucleic acid	55
145	1D3 Light variable + constant - protein	
140	1F3 Heavy Variable + IgG1 constant - nucleic acid	
147	1F3 Heavy variable + IgG1 constant - protein	
148	1F3 Light Variable + constant - nucleic acid	
149	11.5 Light variable + constant - protein 2012 Heavy Variable + IgG1 constant - public acid	10
150	3A12 Heavy Variable + IgG1 constant - Iniciele acid	40
151	2 A 12 Light Variable + apagtant - protein	
152	2 A 12 Light Variable + constant - nucleic acid	
155	5A12 Light variable + constant - protein	

Example 3

Production of Various Recombinant hHGF Proteins

This Example describes the cloning and expression of a number of recombinant proteins used to characterize the antibodies created in Example 1 and in Example 14. In particular, this Example describes the cloning and expression of recombinant hHGF protein, a recombinant hHGF protein contain- 55 ing a glycine to glutamate substitution at position 555 (G555E), a recombinant hHGF protein containing a cysteine to arginine substitution at position 561 (C561R), a recombinant mouse-human-mouse (mhm) chimeric HGF protein containing the human V495-L585 HGF sequence disposed within mouse HGF sequence, a recombinant mhm chimeric HGF protein containing the human I499-R566 HGF sequence disposed within mouse HGF sequence, and a recombinant mhm chimeric HGF protein containing human 65 W507-L585 HGF sequence disposed within mouse HGF sequence.

The following expression constructs were generated using standard molecular techniques and the resulting cDNA sequences were confirmed by DNA sequencing:

a. hHGF-Fc

In a first round of PCR, two overlapping PCR fragments were generated introducing a Not I site and encoding a 6×His tag between hHGF and hIgFc. The overlapping PCR fragments served as template in a second round to amplify hHGFhis-IgFc. The resulting fragment was digested by NheI and BamHI and cloned into pcDNA5/FRT (Invitrogen, #35-3014). Then, hHGF was amplified from Invitrogen clone ID: IOH29794 (human HGF cDNA). The sequence was found to correspond to the sequence deposited at the NCBI under accession number NM_000601.4.

(1) 5'hHGF NheI Primer	(SEO	тп	NO	102)
ACTGGCTAGCATGTGGGTGACCAAACTCCT	1950	10	110.	102)
(2) 3' hHGF NotI His Tag Primer				
	(SEQ	ID	NO.	103)
GIGAIGGIGAIGGIGAIGGCGGCCGCAIGACIGIG	GIACC	IIA	AIAIG	r
(3) 5' HisIgFc Primer				
ACTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	(SEQ	ID	NO.	104)
(4) 3' IgFc BamHI Primer				
ACTGGGATCCTCACTATTTACCCGGGGACAG	(SEQ	ΤD	NO.	105)

hHGF-Fc mutants G555E and C561R were generated by site directed mutagenesis using the QuikChange II XL sitedirected mutagenesis kit (Stratagene) according to manufacturer's instructions.

(1)	hHGF-Fc	(G555E)	Sense Prime:	r FOID NO	100
CAT	GATGTCCAC	GAAAGAG	GAGATGAG	EQ ID NO.	106)
(2)	hHGF-Fc	(G555E)	Anti-sense	Primer FO ID NO	107)
CTC	АТСТССТСІ	TTCGTGG	ACATCATG	LQ ID NO.	107,
(3)	hHGF-Fc	(C561R)	Sense Prime:	r EO ID NO.	108)
GGA	AGAGGAGAI	GAGAAAC	GCAAACAGGTTC	TCAATG	,
(4)	hHGF-Fc	(C561R)	Anti-sense (S	Primer EQ ID NO.	109)
CAT	TGAGAACCI	GTTTGCG	TTCTCATCTCC	TCTTCC	

The mouse-human-mouse chimera IgFc construct contains mHGF alpha chain-hHGF, β-chain amino acids Val 495-Leu 585 of human HGF, and mHGF C-terminal beta chain followed by 6×His tag and IgG-Fc.

Human HGF cDNA encoding amino acids V495-L585 was amplified from Invitrogen clone ID: IOH29794 (human HGF cDNA). The sequence corresponds to the sequence deposited at the NCBI under accession number NM_000601.4. Mouse HGF sequences were amplified by RT-PCR from mouse liver total RNA (Clontech, # 636603) using the Super Script One Step RT-PCR kit from Invitrogen (#10928-034) according to manufacturer's instructions. The mHGF cDNA sequence corresponds to the sequence deposited at the NCBI under accession number D10213.1.

Three fragments, referred to as Fragments 1, 2, and 3, were generated using overlapping PCR primers and annealed in consecutive rounds of PCR amplification. The final product was cleaved with NheI and NotI and cloned into pcDNA5/ FRT IgGFc.

55

(1)	Fragment	1	Primers	for	mHGF	alpha	ch	ain	5'1	IheI
						(S	EQ	ID	NO.	110
51	ATCCCCTAC	~ <u>~</u>	TGATGTGG	CCA	CAAAC	n				

(SEO ID NO. 111) 3 ' GAATCCCATTTACAACCCGCAGTTGTTTTGTTTTGG

(2) Fragment 2 Primers for hHGF beta chain aa V495-L585

5' CCAAAACAAAACAACTGCGGGTTGTAAATGGGATTC

(SEO ID NO. 113) 3 ' CAGGATTGCAGGTCGAGCAAGCTTCATTAAAACCAGATCT

(3) Fragment 3 Primer for mHGF beta chain Cterminus 3'NotI

(SEQ ID NO. 114) 5' AGATCTGGTTTTAATGAAGCTTGCTCGACCTGCAATCCTG

(SEQ ID NO. 115)

3' GTAATTTTGACATACAAGTTGTGCGGCCGCCATCACCATCACCATCA 20 С

d. Construction of hHGF and mhm Chimera

The vectors encoding hHGF and mhm chimera (V495-L585), pcDNA5/FRT hHGF and pcDNA5/FRT-mhm chimera (V495-L585), without Fc-tag were generated by site 25 directed mutagenesis. A stop codon was introduced 3' of the 6×His tag using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. The mutagenesis primer included Primer 1: CATCACCATCACCATCAC-

TAAGCGGGTCTGGTGCCACG (SEQ ID NO. 116), and Primer 2: CGTGGCACCAGACCCGCTTAGTGATGGT-GATGGTGATG (SEQ ID NO. 117).

In addition, two additional mhm chimeras were created from the pcDNA5/FRT-mhm (V495-L585) construct by site 35 directed mutagenesis using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. One mhm construct contained the region of I499-R556 of hHGF disposed between murine sequences. The other mhm construct contained the region of W507-L585 40 of hHGF disposed between murine sequences.

For the mhm chimera (I499-R556), the following point mutations were made in order in the template pcDNA5/FRTmhm chimera (V495-L585) construct: D558E, C561R, V564I, V567I and M583L, using the appropriate oligonucle- 45 teins analyzed by ELISA and by surface plasmon resonance. otide sequences. For the mhm chimera (W507-L585), the following point mutations were introduced in one step in the template pcDNA5/FRT-mhm chimera (V495-L585) construct: Q502R, N₅₀₄T and I505V, using the appropriate oligonucleotide sequences. 50

The resulting nucleotide sequence of the hHGF-Fc protein is set forth as SEQ ID NO. 118, including signal sequence (nucleotides 1-93) and prodomain (nucleotides 94-162). The amino acid sequence of the hHGF-Fc protein is set forth as SEQ ID NO. 119.

The resulting nucleotide sequence encoding the mhm (V495-L585)-Fc chimeric protein is set forth in SEQ ID NO. 120, including signal sequence (nucleotides 1-96) and prodomain (nucleotides 97-165). The amino acid sequence of the mhm (V495-L585)-Fc chimeric protein is set forth in SEQ ID 60 NO. 121.

The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (V495-L585) construct are set forth in SEQ ID NOS. 211 and 212, respectively. The nucleic acid sequence set forth in SEQ ID NO. 211 includes 65 the signal sequence (nucleotides 1-96) and the prodomain (nucleotides 97-165), and the protein sequence set forth in

SEQ ID NO. 212 includes the active protein sequence (without the signal sequence or the prodomain). The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (I499-R556) construct are set forth in SEQ ID NOS. 213 and 214, respectively. The nucleic acid sequence set forth in SEQ ID NO. 213 includes the signal sequence (nucleotides 1-96) and the prodomain (nucleotides 97-165), and the protein sequence set forth in SEQ ID NO. 214 includes the active protein sequence (without the signal (SEQ ID NO. 112) 10 sequence or the prodomain). The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (W507-L585) are set forth in SEQ ID NOS. 215 and 216, respectively. The nucleic acid sequence set forth in SEQ ID NO. 215 includes the signal sequence (nucleotides 1-96) 15 and the prodomain (nucleotides 97-165), and the protein sequence set forth in SEQ ID NO. 216 includes the active protein sequence (without the signal sequence or the prodomain).

e. Protein Expression

(1) Cell Culture

CHO FlpIn cells (Invitrogen, Catalog No. R758-07)) were grown in F12K media (ATCC, Catalog No. 30-2004), 10% FCS (Invitrogen, Catalog No. 10438026), 1% Penicillin (10000 units/mL)/Streptomycin (10,000 µg/mL) (Invitrogen, Catalog No. 15140-122) at 37° C., 5% CO2, 100 µg/mL Zeocin (Invitrogen, Catalog No. R250-01).

(2) Generation of Stable CHO FlpIn Cell Lines

CHO FlpIn host cells were transfected with a 9:1 ratio of pOG44:pcDNA5/FRT expression plasmid DNA using lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Catalog No. 11668-027). As controls, cells were transfected with empty pcDNA5/FRT vector/pOG44 and pOG44 plasmid (Invitrogen, Catalog No. 35-3018) alone. Twenty four hours after transfection, the cells were split, and after forty eight hours 0.5 mg/mL Hygromycin B (Sigma, Catalog No. H0654-SPEC) was added to the cells. Polyclonal selection of stable cells was performed in F12K, 10% FCS, 1% Penicillin/Streptomycin, 0.5 mg/mL Hygromycin B.

(3) Protein Expression in Stable CHO FlpIn Cell Lines

Approximately 2×10^6 cells were seeded in 15 cm plates and grown in F12K (ATCC, Catalog No. 30-2004)/DMEM high.glucose (Invitrogen, Catalog No. 11995065) 1:1, 5% ultra low IgG FCS (Invitrogen, #16250-78) at 37° C., 5% CO₂ for 5-6 days. Supernatants were harvested and resulting pro-

Example 4

Binding Characteristics of Anti-hHGF Monoclonal Antibodies

The monoclonal antibodies produced in Example 1 were characterized by their ability to bind hHGF, and certain of the recombinant HGF proteins produced in Example 3.

The antibodies were analyzed by surface-plasmon resonance using a BIAcore T100 instrument to assess their ability to bind HGF and certain of the fusion proteins discussed in Example 3. Each antibody was immobilized on a carboxymethylated dextran CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions.

Analyses were performed at 25° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. R-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer. Supernatant

containing different HGF fusion proteins or supernatant from cells transfected with empty vector were injected over each antibody at a flow rate of 30 µL/min for 3 minutes. The resulting binding was determined as resonance units (RU) over baseline 30 seconds after the end of injection. Binding 5 was compared to human HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer. Non-specific binding was monitored by comparing binding to a control surface where mouse IgG (Rockland, Catalog No. 010-0102) was immobilized using the same amine coupling procedure.

The results are summarized in the Table 3.

TABLE 3

Antibody	rhHGF (R&D Systems)	rmHGF (R&D Systems)	mhm chimera (V495-L585)	human HGF	G555E	C561B
1A3	Yes	No	No	Yes	Yes	Yes
1D3	Yes	No	Yes	Yes	Yes	Yes
1F3	Yes	Yes	Yes	Yes	Yes	Yes
2B8	Yes	No	Yes	Yes	Yes	Yes
2F8	Yes	Yes	No	Yes	Yes	Yes
3A12	Yes	No	No	Yes	Yes	Yes
3B6	Yes	No	No	Yes	Yes	Yes
3D11	Yes	No	No	Yes	Yes	Yes

The results in Table 3 demonstrate that each of the antibodies bind rHGF and purified human HGF. Furthermore, all of the antibodies bind hHGF containing point mutations G555E and C⁵⁶¹R. In general, all of the antibodies except for 1F3 and 2F8 did not bind murine HGF demonstrating that the antibodies 1A3, 1D3, 2B8, 3A12, 3B6, and 3D11 specifically bind human HGF. Antibodies 1D3, 1F3, and 2B8 bind the mouse-human-mouse chimera whereas the remaining antibodies did not. The results suggest that the antibodies 1D3 and 2B8 at least in part bind to residues 495-585 of human HGF. The antibodies 1A3, 3A12, 3B6, and 3D11 appear to bind portions of human hHGF other than residues 495-585. At present, it is uncertain why 2F8 does not bind the mhm chimera as it appears to bind both hHGF and mHGF.

Example 5

Ability of Anti-hHGF Monoclonal Antibodies to Bind Reduced and Non-Reduced HGF

In this Example, the anti-hHGF monoclonal antibodies produced in Example 1 were analyzed for their ability to bind reduced and non-reduced HGF.

The reactivity of the anti-HGF sera with the recombinant $_{50}$ hHGF was assessed by immunoblotting. Eight µg of recombinant hHGF protein in NuPAGE MOPS SDS running buffer (Invitrogen) with or without NuPAGE sample reducing buffer (Invitrogen) was fractionated on a 4-12% Bis-Tris 1.0 mm×2D well gel (Invitrogen, Carlsbad, Calif.). The fraction- 55 ated proteins then were transferred onto a nitrocellulose membrane using standard procedures. The nitrocellulose membranes were blocked with 5% nonfat milk powder solution in Tris buffered Saline with 0.1% Tween-20® (TBST), and then mounted onto a Mini Protean II Multi-Screen appa-60 ratus (BioRad) for further blocking.

The resulting membranes were probed with the purified antibodies on a Multi-Screen apparatus. The purified antibodies were diluted to 5 μ g/mL in blocking buffer. The nitrocellulose membrane then was removed from the apparatus, and incubated with horseradish peroxidase-labeled anti-mouse IgG antibodies. The results are summarized in Table 4, where

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the numbers reflect the extent of binding with-representing the least (little or no binding) and 3+ representing the most binding.

TABLE 4 Reduced Non-Reduced Antibody (exposure: 3-5 min) (exposure: 20 sec) 1A3 2+2+1D3 2+2+

25

45

TABLE 4-continued

Antibody	Reduced (exposure: 3-5 min)	Non-Reduced (exposure: 20 sec)
1F3	2+	2+
2B8	_	1+
2F8	2+	2+
3A12	_	2+
3B6	3+	2+
3D11	—	3+

The data in Table 4 demonstrate that all the antibodies bind non-reduced rhHGF. In contrast, monoclonal antibodies 1A3, 1D3, 1F3, 2F8, 3B6 bound reduced rhHGF but antibodies 40 2B8, 3A12, and 3D11 did not bind to reduced rhHGF.

Example 6

Binding Affinities

The binding affinities and kinetics of interaction of each of the antibodies produced in Example 1 against hHGF were measured by surface plasmon resonance.

Rabbit anti-mouse immunoglobulins (BIAcore, Catalog No. BR-1005-14) were immobilized on carboxymethylated dextran CM5 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. The analyses were performed at 25° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930), and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer.

The antibodies were captured in an individual flow cell at a flow rate of 10 µL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at

 $60\,\mu$ L/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10 mM Glycine-HCl, pH 1.7 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 μ L/min before another cycle was initiated. HGF con-5 centrations tested were 0.46 nM to 7.5 nM.

Kinetic parameters were determined using the kinetic function of the BIAevalutation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equi- 10 librium dissociation constant) are summarized in Table 5.

TABLE 5

Antibody	ka (1/Ms)	SE (ka)	kd (1/s)	SE (kd)	$\begin{array}{c} \mathbf{K}_{D} \\ (\mathbf{p}\mathbf{M}) \end{array}$	SD	15
1A3	1.7×10^{6}	7.3×10^{4}	5.2×10^{-5}	8.4×10^{-7}	30.1	5.6	
1D3	1.7×10^{6}	3.1×10^4	8.2×10^{-5}	1.7×10^{-6}	54.2	27.4	
1F3	1.5×10^{6}	5.0×10^{4}	2.6×10^{-5}	6.6×10^{-7}	18.1	8.2	
2B8	1.6×10^{6}	2.9×10^{4}	2.1×10^{-5}	1.4×10^{-7}	13.5	4.4	
3A12	1.6×10^{6}	3.7×10^4	1.6×10^{-4}	1.6×10^{-6}	103.0	10.4	20
3B6	2.0×10^{6}	6.5×10^{4}	3.9×10^{-5}	3.2×10^{-7}	17.0	3.4	

The data in Table 5 demonstrate that the antibodies bind hHGF with a K_D of about 100 pM or less, about 50 pM or less, or 20 pM or less. ²⁵

Example 7

Neutralization Activity of Anti-hHGF Antibodies

In this Example, the antibodies produced in Example 1 were characterized for their ability to (a) inhibit the binding of hHGF to c-Met, and (b) inhibit HGF stimulated BrdU incorporation in 4 MBr-5 cells.

a. HGF-Met Binding Inhibition Assay (Neutralization 35 Assay)

The antibodies were tested by ELISA for their ability to inhibit hHGF binding to c-Met.

Specifically, Wallac 96-well DELFIA assay plates (Wallac Inc., Catalog No. AAAND-0001) were coated with 100 µL of 40 6.25 µg/mL HGF (R&D Systems, Catalog No. 294-HGN-025) in carbonate coating buffer (15 mM Na₂CO₃ and 34 mM NaHCO₃, pH 9.0) for 16 hours at 4° C. The plates then were blocked with 200 µL of 5% non-fat dry milk in PBS for 1 hour at room temperature. The antibodies were prepared in a sepa- 45 rate plate by adding increasing concentrations of the antibodies under investigation (0.033-667 nM, 3-fold-serial dilution) to 2 nM c-Met (R&D Systems, Catalog No. 358-MT/CF) in 5% non-fat dry milk in PBS. 100 µL of sample per well was transferred to the assay plate and incubated overnight at 4° C. 50 The assay plates then were washed 3 times with PBS-0.1% Tween 20, and incubated for 2 hours at room temperature with 100 µL/well of 2 µg/mL biotinylated anti-human c-Met antibody (R&D Systems, Catalog No. BAF358) prepared in 5% non-fat dry milk in PBS.

The resulting plates then were washed three times with PBS-0.1% Tween 20, and incubated for 1 hour at room temperature with Eu-labeled Streptavidin (Wallac, Catalog No. 1244-360) diluted 1:1000 in DELFIA assay buffer (Wallac, Catalog No. 4002-0010). The resulting plates were washed 3 6 times with DELFIA wash solution (Wallac, Catalog No. 4010-0010) and incubated with 100 μ L/well DELFIA enhancement solution (Wallac #4001-0010) for 15 minutes at room temperature with agitation.

The plates were read on Victor³V instrument (Perkin 65 Elmer) using the Europium method. The IC_{50} values were calculated and are summarized in Table 6.

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TABLE 6

Antibody	$IC_{50}\left(nM\right)$	SD	
1A3	5.65	0.91	
1D3	4.43	2.27	
1F3	6.57	0.28	
2B8	5.57	1.19	
2F8	5.36	0.88	
3A12	5.26	2.11	
3B6	_	—	
3D11	5.66	2.75	

The results demonstrate that all the antibodies (i.e., 1D3, 1A3, 2B8, 3A12, 1F3, 3D11, and 2F8) other than 3B6 efficiently neutralize HGF binding to c-Met.

b. Neutralization of HGF Stimulated BrdU Incorporation in 4 MBr-5 Cells

Ten μ L of 12.5 nM of hHGF was dispensed into individual wells of a 96-well tissue culture microtiter plate (Costar Catalog No. 3903). Ten μ L of serially diluted antibodies at concentrations of 6667, 2222, 740, 247, 82, 27, 9.1, 3.0, 1.0, 0.33 nM were added to each well. The HGF antibody mixture then was incubated at room temperature for 30 minutes. Monkey bronchial epithelial cells 4 MBr-5 (ATCC, CCL208) cultured in F-12K (ATCC, 30-2004), 15% FBS (Gibco 10438-026), 30 ng/mL EGF (Sigma E9644), 1% penicillin/streptomycin (PS, Gibco Catalog No. 15140-122) were dissociated with Trypsin (Gibco Catalog No. 25200-056), resuspended in assay media (F-12K, 2.5% FBS, 1% PS) at 75,000 cells/mL, and 80 μ L of the cell suspension was dispensed to the HGF antibody mixture.

The resulting cells were incubated at 37° C., 5% CO₂. Forty eight hours later, $10 \,\mu$ L of $100 \,\mu$ M BrdU (Roche Catalog No. 1669915) was added. Seventy two hours later, the media was removed, the plates were dried with a hair dryer and were processed with the BrdU ELISA in accordance with manufacturer's instructions (Roche Catalog No. 1669915).

The luminescent signal was quantified by a Synergy HT plate reader (Bio-Tek). The data were fit to a sigmoidal dose response with variable slope with the equation y=bottom+(top-bottom)/(1+10^(log(EC50-x)*hill slope)) in GraphPad Prism (GraphPad Software). Each experiment was repeated at least 3 times in duplicates, and average EC_{50} values are presented in Table 7.

TABLE 7

5	Antibody	$\mathrm{IC}_{50}\left(nM\right)$	
	1A3	4.69	
	1D3	4.99	
	1F3	1.94	
	2B8	1.41	
0	2F8	19.24	
0	3A12	30.30	
	3B6	36.08	
	3D11	51.12	

The results in Table 7 demonstrate that all of the antibodies, 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6, and 3D11 inhibit HGF induced proliferation in 4 MBr-5 cells.
- 5

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Example 8

Anti-Scatter Activity of Anti-hHGF Antibodies

This Example describes a characterization of the antibodies produced in Example 1 for their ability to inhibit HGF induced scatter activity. HGF induces "scattering" (motility) of clusters in MDCK cells (ATCC, Manassas, Va., Catalog No. CCL-34).

10MDCK cells were seeded in 96-well Costar tissue culture plates (Corning Incorporated, Corning, N.Y., Catalog No. 3595) at a density of 4×10^3 cells per well in 80 µL MEM (ATCC, Manassas, Va., Catalog No. 30-2003) containing 10% Fetal Bovine Serum (Invitrogen Catalog No. 10438026), and 1% penicillin-streptomycin (Invitrogen Catalog No. 15140122). Each of the antibodies to be investigated was diluted to 6,667 nM in MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin. Each of the different antibody dilutions, as well as MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin without antibody, then was separately combined with an equal volume of MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin, and 100 ng/ml HGF (R&D Systems Catalog No. 294-HGN-025). The antibody/HGF dilutions were 25 incubated for 30 minutes at 25° C. Twenty µL of each antibody/HGF dilution was added separately to individual wells, yielding a final antibody concentration of 666.7 nM, and a final HGF concentration of 10 ng/ml. The MDCK cells then were incubated for 24 hours at 37° C. with 5% CO₂.

After 24 hours incubation, the MDCK cells were carefully washed once with 100 µL per well of ice-cold PBS (Invitrogen Catalog No. 14190144), and fixed with 100 µL per well of ice-cold methanol while rocking for 10 minutes at 25° C. The plates then were washed carefully once with distilled water. A volume of 100 µL crystal violet solution, consisting of 0.5% crystal violet (Sigma, St. Louis, Mo., Catalog No. C3886) and 50% ethanol in distilled water, was added to each well, and the cells were incubated for 20 minutes at 25° C. while rocking

Following staining with crystal violet solution, the cells were washed carefully three times with distilled water. Then, PBS was added to each well to prevent drying of samples. The cells were imaged using the Leica DMIRB microscope (Leica Microsystems GmbH, Wetzler, Germany), DC500 camera 45 (Leica Microsystems GmbH, Wetzler, Germany), and MagnaFire 2.1C software (Optronics, Goleta, Calif.), and samples were rated for level of scattering. The results are summarized in Table 8.

TABLE 8

Inhibition o	of HGF-induced MD Scattering	CK Cell	
Antibody	Trial 1	Trial 2	55
1A3	++	+	
1D3	++	++	
1F3	+	+	
2B8	+++	+++	
2F8	+	+	60
3A12	-	-/+	60
3B6	++	++	
3D11	-	-	

- No Inhibition

+++ Very strong, nearly complete inhibition

++ Strong inhibition

+ Detectable inhibition

The results in Table 8 demonstrate that antibody 2B8 inhibited HGF-induced scattering more than the other antibodies. Antibodies 1D3 and 3B6 displayed an intermediate level of inhibition; antibody 1A3 displayed a low to intermediate level of inhibition: antibodies 1F3 and 2F8 displayed a low level of inhibition; and antibodies 3A12 and 3D11 gave little or no detectable inhibition.

Example 9

Inhibition of HGF-Stimulated c-Met Phosphorylation

This Example describes a characterization of the antibod-15 ies produced in Example 1 for their ability to inhibit the HGF-stimulated c-Met phosphorylation in PC-3 cells. HGF induces phosphorylation of Met in PC-3 cells (ATCC No. CRL-1435).

PC-3 cells were seeded into individual wells of 96-well Costar tissue culture plates (Corning Catalog No. 3595) at a density of 4.5×10^4 cells per well in 100 µL F-12K (ATCC, Manassas, Va., Catalog No. 30-2004) containing 10% Fetal Bovine Serum (Invitrogen Catalog No. 10438026) and 1% penicillin-streptomycin (Invitrogen Catalog No. 15140122). After 24 hours at 37° C. with 5% CO₂, the media was removed, and cells were rinsed once with serum-free F-12K containing 1% penicillin-streptomycin. Cells then were incubated for 24 hours in 100 µL serum-free F-12K containing 1% penicillin-streptomycin.

The following 10 different dilutions of each of the antibodies being investigated were prepared in serum-free F-12K containing 1% penicillin-streptomycin: 6667 nM, 2222 nM, 741 nM, 247 nM, 82.3 nM, 27.4 nM, 9.1 nM, 3.0 nM, 1.0 nM, and 0.3 nM. Each antibody dilution, and, serum-free F-12K 35 containing 1% penicillin-streptomycin without antibody, were separately combined with an equal volume of serumfree F-12K containing 1% penicillin-streptomycin and 500 ng/mL HGF (R&D Systems Catalog No. 294-HGN-025). These antibody/HGF dilutions were incubated for 30 minutes $_{40}\,$ at 25° C. This resulted in a final concentration of 1.25 nM HGF.

The PC-3 cells then were rinsed once with serum-free F-12K containing 1% penicillin-streptomycin. Next, 70 µL of serum-free F-12K containing 1% penicillin-streptomycin was added to the cells, followed by $10 \,\mu\text{L}$ of $10 \,\text{mM} \,\text{Na}_3 \text{VO}_4$ (Sigma Catalog No. S6508) in serum-free F-12K containing 1% penicillin-streptomycin. The cells then were incubated for 60 minutes at 37° C. with 5% CO₂. Following this incubation, 20 µL of each antibody/HGF dilution was added sepa-50 rately to separate wells, yielding a final HGF concentration of 50 ng/mL, and the following final concentrations of each antibody: 666.7 nM, 222.2 nM, 74.1 nM, 24.7 nM, 8.23 nM, 2.74 nM, 0.91 nM, 0.30 nM, 0.10 nM, 0.03 nM. The cells then were incubated for 10 minutes at 37° C. with 5% CO₂, after which point the media/antibody/HGF mixture was removed, the plates were placed on ice. The cells then were rinsed once with 100 µL per well of ice-cold PBS (Invitrogen Catalog No. 14190144) containing 1 mM Na_3VO_4 . The cells then were incubated for 30 minutes at 4° C. in 100 µL per well ice-cold lysis buffer consisting of 1% OmniPur Triton X-100 (MERCK KGaA, Darmstadt, Germany, Catalog No. 9410), 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.3 mM Na₃VO₄, 1× protease inhibitor cocktail (Sigma Catalog No. P8340), and 1× phosphatase inhibitor cocktail 2 (Sigma Catalog No. 65 5726).

Biotinylated anti-human HGF-R (c-met) antibody (R&D Systems Catalog No. BAF358) was diluted to a concentration

of 2 µg/mL in DELFIA Assay Buffer (PerkinElmer, Turku, Finland, Catalog No. 4002-0010) containing 1% bovine serum albumin (Sigma Catalog No. A2153), and 50 µL of this dilution was added per well of yellow streptavidin microtitration plates (PerkinElmer Catalog No. AAAND-0005). The 5 plates then were incubated with antibody for 30 minutes at 25° C. with rocking. Following incubation, the plates were washed with DELFIA wash solution (PerkinElmer Catalog No. 4010-0010), and $80 \,\mu\text{L}$ of each of the different PC-3 cell lysates was added separately to individual wells of the 10 washed streptavidin microtitration plates.

The streptavidin microtitration plates containing PC-3 cell lysates were incubated for 60 minutes at 25° C. with shaking, and then washed with DELFIA wash solution. $100 \,\mu\text{L}$ of 600ng/mL DELFIA Eu-NI P-Tyr-100 antibody (PerkinElmer 15 Catalog No. AD0159) diluted in DELFIA Assay Buffer containing 1% bovine serum albumin was added to each well of the washed streptavidin microtitration plates previously incubated with PC-3 cell lysates. The plates were incubated for 60 minutes at 25° C., with rocking. The plates were washed a 20 final time with DELFIA wash solution. Then 200 µL of DELFIA Enhancement Solution (PerkinElmer Catalog No. 4001-0010) was added to each well of the washed streptavidin microtitration plates, and the plates were incubated in the dark for 5 minutes at 25° C., with shaking.

Signal then was measured using the Europium protocol on the Victor3V reader (PerkinElmer). EC50 values were calculated using Prism 4 for Windows (GraphPad Software, Inc., San Diego, Calif.) and the sigmoidal dose-response equation.

The results summarized as EC50s in nM are tabulated in 30 Table 9.

TABLE 9

3	Standard Deviation	Average of Two Trials	Antibody
	0.242	0.684	1A3
	0.129	0.984	1D3
	1.01	1.19	1F3
	0.104	0.287	2B8
40	2.12	1.39	2F8
	0.553	2.00	3A12
	1.11	1.01	3B6
	N/A	2.28	3D11

The data in Table 9 demonstrate that all eight antibodies are 45 potent inhibitors of HGF-induced c-Met phosphorylation in PC-3 cells.

Example 10

Tumor Inhibition in U87MG Xenograft Model

The ability of murine monoclonal antibodies of the invention to inhibit tumor growth was tested in an U87MG xenograft model. U87MG cells (ATCC) were expanded in 55 culture at 37° C. in an atmosphere containing 5% CO2 and 95% air, using a medium comprising Dulbecco's Modified Eagle medium (DMEM) with 10% fetal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were subcultured and maintained by detaching the cells from 60 the wall of the culture dish using trypsin-EDTA.

Near-confluent cells were collected by trypsinization and then 5×10⁶ cells in 50% Matrigel (BD Biosciences; catalog no. 356237) were injected subcutaneously into the upper dorsal area between the shoulder blades of 7-week old female 65 ICR SCID mice (Taconic Labs). The long (L) and short (W) diameters (mm) of tumors were measured with a caliper.

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Tumor volume (vol.) was calculated as: volume (mm³)=L× $W^2/2$. When the tumors grew to approximately 200 mm³, the tumor-bearing mice were randomized into 5 groups of 10 mice each. One group received PBS. Each of the other 4 groups received one of the antibody 1A3, 1D3, 1F3 or 2B8. All antibodies were dosed at 1 mg/kg body weight, twice per week, by intra-peritoneal injections of 5 doses. Tumor volumes and mouse body weights were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test. The results are summarized in FIG. 6 and Table 10.

TABLE 10

	Percent Inhibition				
2B8 vs PBS	93%	p = 0.001			
1A3 vs PBS	73%	p = 0.0075			
1D3 vs PBS	51%	p = 0.075			
1F3 vs PBS	60%	p = 0.027			

Partial regression was achieved in 2B8 treated group (FIG. 6). Statistically significant growth inhibition was observed in the 1A3-treated and 1F3-treated groups (Table 10). There was 51% tumor growth inhibition for 1D3 with a p value of 0.075. No significant body weight loss was observed.

Example 11

Tumor Inhibition in U118 Xenograft Model

The ability of the antibodies 1A3, 1D3, 1F3 and 2B8 to inhibit tumor growth was tested in an U118 xenograft model. U118 cells (ATCC) were expanded as described in Example 10 (above) with respect to the U87MG cells.

Subcutaneous tumors were established as described in 5 Example 10 above, except that the mice used were 7 weeks old female NCr nude mice (Taconic), and treatment was started when the tumors grew to approximately 80 mm³. As in the U87MG model, all the antibodies were dosed at 1 mg/kg body weight twice a week by intra-peritoneal injections for 4 doses. Tumor volumes and body weights of the mice were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test. The results are summarized in FIG. 7 and Table 11.

TABLE 11

		Percent Inhibition	
	2B8 vs IgG	75%	p = 0.007
	1A3 vs IgG	57%	p = 0.01
50	1D3 vs IgG	47%	p = 0.12
50	1F3 vs IgG	30%	p = 0.39

Statistically significant tumor growth inhibition was observed in 2B8 and 1A3 treated groups (FIG. 7). There was modest tumor growth inhibition in 1F3 and 1D3 groups with p values less than 0.05, which was defined as statistical significance in this study (Table 11). No significant body weight loss was observed.

Example 12

Humanization of Murine Monoclonal Antibodies

This Example describes the humanization of the murine 2B8 antibody, together with a characterization of the resulting humanized antibodies. The murine 2B8 Heavy and Light Variable Regions were "humanized" by two methods.

A. Humanization Procedure 1

In the first method, three humanized heavy chain variable regions and two humanized kappa light chain variable regions were designed based on the "superhumanization" method described in Hwang et al. (2005) METHODS 36:35-42; Tan et al. 5 (2002) J. IMMUNOL 169:1119-1125; U.S. Pat. No. 6,881,557.

The Chothia canonical structural class was determined for each mouse 2B8 CDR based on CDR length and amino acid composition. Human germline variable regions consisting of the same Chothia canonical structural class light and heavy 10 variable regions were identified based on known human germline variable region reference alleles described at the International Immunogenetics Information System (IMGT) website (available on the world wide web at imgt.cines.fr and biochem.unizh.ch/antibody/Sequences/index.html). These 15 human germline variable regions of the same structural class were compared to murine 2B8 variable regions by calculating the percent identity or similarity between CDR amino acid residues. Those human germline variable regions with the highest identity and/or similarity with mouse 2B8 CDR resi- 20 dues were chosen for CDR grafting. The framework residues of the human germline variable regions were preserved while the mouse 2B8 CDR residues were used to replace the corresponding human germline variable region residues that were different between mouse 2B8 CDR and human germline 25 CDRs. The human J region that was most similar to the 2B8 mouse J region was then added to the carboxyl terminus of the "superhumanized" variable region. A signal sequence was then added to the amino terminus of the "superhumanized" variable regions and these amino acid sequences were con- 30 verted into nucleic acid sequences.

The complete variable region nucleic acid sequence was constructed using gene synthesis PCR methods (Young et al. (2004) NUCL. ACIDS RES. 32:e59) and cloned into a mammalian expression vector (based on pcDNA3.2 DEST (Invitrogen)) 35 containing human constant IgG1 (G1m(17,1) allotype) or Kappa (Km(3) allotype (allele 2)) regions (downstream of the variable regions) using standard molecular biology techniques. All four heavy chain IgG1 antibodies (chimeric 2B8 and 3 humanized heavy chains (Hu2B8 Hv1-f.1, Hu2B8

Hv5-a.1, Hu2B8 Hv5-51.1) were expressed in the possible combinations with all 3 kappa chain antibodies (chimera 2B8 and 2 humanized light chains (Hu2B8 Kv1-39.1 and Hu2B8 Kv3-15.1) creating 12 different antibody proteins. Binding of the chimeric, chimeric/humanized, and humanized antibodies to human HGF was then measured as described below and the results are summarized in FIG. **8**. Each of the possible combinations of immunoglobulin heavy chain and immunoglobulin light chain variable regions are set forth below in Table 12A.

TABLE 12A

Heavy Chain Variable Region	Light Chain Variable Region
Chimeric 2B8 (SEQ ID NO: 12) Chimeric 2B8 (SEQ ID NO: 12)	Chimeric 2B8 (SEQ ID NO: 14) Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Chimeric 2B8 (SEQ ID NO: 12)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)

Each of the possible combinations of immunoglobulin heavy chains and immunoglobulin light chains are set forth below in Table 12B.

TABLE 12B

Immunoglobulin Light Chain
Chimeric 2B8 Kappa (Km(3)) (SEO ID NO: 157)
Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEO ID NO: 177)
Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)
Chimeric 2B8 Kappa (Km(3))
(SEQ ID NO: 157)
Hu2B8 Kv1-39.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Kv3-15.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 181)
Chimeric 2B8 Kappa (Km(3))
(SEQ ID NO: 157)
Hu2B8 Kv1-39.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Kv3-15.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 181)
Chimeric 2B8 Kappa (Km(3))
(SEQ ID NO: 157)
Hu2B8 Kv1-39.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Kv3-15.1 + Kappa Constant (Km(3)
allotype) (allele 2) (SEQ ID NO: 181)

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Two of the possible antibody constructs containing the full length immunoglobulin heavy and light chains containing humanized variable regions are designated below:

- sh2B8-9 (G1m(17,1))=hu2B8 Hv5-51.1 (+IgG1 constant region (G1m(17,1) allotype) (SEQ ID NO. 171) plus hu2B8 Kv 1-39.1 (+Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 177)
- sh2B8-12 (G1m(17,1))=hu2B8 Hv5-51.1 (+IgG1 constant region (G1m(17,1) allotype)) (SEQ ID NO. 171) plus ¹⁰ hu2B8 Kv 3-15.1 (+Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 181).

The nucleic acid sequences encoding and the protein sequences defining each of the humanized antibodies are summarized below. In this section, the last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region junction. This nucleotide is included in the Variable Region because it is part of that exon. Amino acid sequences of Constant Regions listed below 20 include the translation of this junction codon.

(1) Nucleic Acid Sequence Encoding the Full Length Chimeric 2B8 Heavy Chain (Mouse Variable Region and Human IgG1 Constant Region) (allotype Glm(17, 1)) (signal sequence underlined) (SEQ ID NO. 154)

- 1 <u>atgggatgga gctatatcat cctctttttg gtagcaacag</u> <u>ctacagatgt ccactcc</u>cag
- 61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
- 121 tgcaaggett etggetacae etteaceaee taetggatge aetgggtgaa teagaggeet
- 181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
- 241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg
- 301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
- 361 ggtagcatet ttgactaetg gggecaagge accaetetea ceqteteete ageeteeae
- 421 aagggeeeat eggtetteee eetggeaeee teeteeaaga geaeetetgg gggeaeageg
- 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
- 541 ggegeeetga eeageggegt geacacette eeggetgtee tacagteete aggaetetae
- 601 teeeteagea gegtggtgae egtgeeetee ageagettgg geaeceagae etacatetge
- 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt
- 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
- 781 tteetettee eeccaaaace eaaggacace etcatgatet eeeggaceee tgaggteaca
- 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
- 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
- 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag

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- 1021 tgcaaggtet ccaacaaage ecteecagee eccategaga aaaceatete caaageeaaa
- 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag
- 1141 aaccaggtca geetgaeetg eetggteaaa ggettetate eeagegaeat egeegtggag
- 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
- 1261 gacggeteet tetteeteta cagcaagete acegtggaca agageaggtg geageagggg
- 1321 aacgtettet eatgeteegt gatgeatgag getetgeaca accaetaeae geagaagage
- 1381 ctctccctgt ctccgggtaa atga

(2) Protein Sequence Defining the Full Length Chimeric 2B8 Heavy Chain (Chimeric 2B8 IgG1

- (Glm(17, 1) allotype) (without signal sequence) (SEQ ID NO. 155)
 - 1 qvqlqqpgae lvkpgtsvkl sckasgytft tywmhwvnqr pgqglewige inptnghtny
 - 61 nekfkskatl tvdkssstay mqlssltsed savyycarny vgsifdywgq gttltvssas
 - 121 tkgpsvfpla psskstsggt aalgclvkdy tpepvtvswn sgaltsgvht fpavlqssgl
 - 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps
 - 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 - 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt
- 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
- 421 gnvfscsvmh ealhnhytqk slslspgk

(3) Nucleic Acid Sequence Encoding the Full Length Chimeric 2B8 Light Chain (Mouse Variable Region 45 and Human Constant Region) (Chimeric 2B8 Kappa

- (Km(3))) (signal sequence underlined) (SEQ ID NO. 156)
 - 1 <u>atggaatcac agactetggt etteatatee atactgetet</u> <u>ggttatatgg tgetgatggg</u>
 - 61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
 - 121 ttgagetgea aggeeagtga gaatgtggtt tettatgtat eetggtatea acagaaacea
 - 181 gcgcagtctc ctaaactgct gatatacggg gcatccaacc ggaacactgg ggtccccgat
 - 241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
 - 301 gaagacettg cagattatea etgtgggeag agttacaaet ateegtacae gtteggaggg
 - 361 gggaccaggc tggaaataaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca
 - 421 tetgatgage agttgaaate tggaactgee tetgttgtgt geetgetgaa taaettetat

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- 481 cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcqqq taactcccaq
- 541 gagagtgtca cagagcagga cagcaaggac agcacctaca qcctcaqcaq caccctqacq
- 601 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
- 661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttga

(4) Protein Sequence Defining the Full Length
Chimeric 2B8 Light Chain (Chimeric 2B8 Kappa (Km(3))) (without signal sequence) (SEQ ID NO.
157)

- 1 nivmtqspks msmsvgervt lsckasenvv syvswyqqkp aqspklliyg asnrntgvpd
- 61 rftgsgsatd ftltissvra edladyhcgq synypytfgg gtrleikrtv aapsvfifpp
- 121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
- 181 lskadyekhk vyacevthqg lsspvtksfn rgec

(5) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv1-f.1 Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 158)

- 1 <u>atggactgca cctggaggat cctcctcttg gtggcagcag</u> <u>ctacaggcac ccacgccg</u>ag
- 61 gtccagctgg tacagtctgg ggctgaggtg aagaagcctg
 qqqctacaqt gaaaatctcc
- 121 tgcaaggttt ctggatacac cttcaccacc tactggatgc actgggtgca acaggcccct
- 181 ggaaaagggc ttgagtggat gggagagatt aatcctacca acggtcatac taactacaat
- 241 gagaagttee agggeagagt eaceataace geggaeaegt etaeagaeae ageetaeatg
- 301 gagetgagea geetgagate tgaggaeaeg geegtgtatt aetgtgeaae aaaetatgtt
- 361 ggtagcatet ttgactaetg gggeeaagga accetggtea eegteteete ag

-continued

- (6) Protein Sequence Defining Humanized Hu2B8
- Hv1-f.1 Heavy Chain Variable Region (without signal sequence)
- (SEQ ID NO. 159)
 - 1 evqlvqsgae vkkpgatvki sckvsgytft tywmhwvqqa pgkglewmge inptnghtny
- 61 nekfqgrvti tadtstdtay melsslrsed tavyycatny vgsifdywgq gtlvtvss
- (7) Nucleic Acid Sequence Encoding Human IgG1 Heavy Chain Constant Region (Glm(17, 1) allotype) (SEQ ID NO. 160)
- 1 cctccaccaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg
- 61 gcacageggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg acggtgtcgt
- 121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag
- 181 gactetacte eetcageage gtggtgaeeg tgeeeteeag cagettggge acceagaeet
- 241 acatetgeaa egtgaateae aageeeagea acaeeaaggt ggacaagaaa gttgageeea
- 301 aatettgtga caaaaeteae acatgeeeae egtgeeeage acetgaaete etgggggggae
- 361 cgtcagtett cetetteece ccaaaaeceea aggacaecet catgatetee eggaceeetg
- 421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
- 481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
- 541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
- 601 agtacaagtg caaggtetee aacaaageee teecageeee categagaaa aceateteea
- 661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggatgagc
- 721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
- 781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc
- 841 tggacteega eggeteette tteetetaea geaageteae egtggacaag ageaggtgge
- 901 agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc
 - 961 agaagageet eteestgtet eegggtaaat ga
- ⁵⁵ The first amino acid is derived from translation of the last nucleotide of variable region and beginning two nucleotides of the IgG1 Heavy Chain sequence.

(8) Protein Sequence Defining Human IgG1 Heavy Chain Constant Region $({\tt Glm}\,(17\,.1)$ allotype).

(SEQ ID NO. 161) 1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss

61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkkvep kscdkthtcp pcpapellgg

121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreegyn

-continued 181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsrde 241 ltknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw

301 ggqnvfscsv mhealhnhyt gkslslspgk

61 gtccagctgg tacagtctgg ggctgaggtg aagaagcctg gggctacagt gaaaatctcc 121 tgcaaggttt ctggatacac cttcaccacc tactggatgc actgggtgca acaggcccct 181 ggaaaagggc ttgagtggat gggagagatt aatcctacca acggtcatac taactacaat 241 gagaagttee agggeagagt caccataace geggacaegt etacagacae ageetacatg 301 gagetgagea geetgagate tgaggaeaeg geegtgtatt aetgtgeaae aaaetatgtt 361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc 421 aagggeecat eggtetteee eetggeacee teeteeaaga geacetetgg gggeacageg 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 601 teectcagea gegtggtgae egtgeeetee ageagettgg geacecagae etacatetge 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tectgggggg accgtcagtc 781 tteetettee eeccaaaace caaggacace etcatgatet eeeggaceee tgaggteaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtet ecaacaaage esteecagee eccategaga aaaccatete caaagecaaa 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1261 gacggeteet tetteeteta cagcaagete accgtggaca agagcaggtg geageagggg 1321 aacgtettet catgeteegt gatgeatgag getetgeaca aceaetaeae geagaagage 1381 ctctccctgt ctccgggtaa atga

(10) Protein Sequence Defining the Full Length Heavy Chain Humanized Hu2B8 Hv1f.1 Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(17,1) allotype) (without signal sequence)

(SEQ ID NO. 163) 1 evqlvqsgae vkkpgatvki sckvsgytft tywmhwvqqa pgkglewmge inptnghtny 61 nekfqgrvti tadtstdtay melsslrsed tavyycatny vgsifdywgq gtlvtvssas 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq 421 gnvfscsvmh ealhnhytgk slslspgk 64

(SEO ID NO. 164)

-continued

(11) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region (signal sequence underlined)

<u>atgqqqtcaa ccqccatct cqcctctc ctqqctqttc tccaaqqaqt ctqtqccqaa</u>
 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaggatctcc
 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
 ccgtccttcc aaggccacgt caccatctca gctgacaagt ccatcagcac tgcctacctg
 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
 ggtagcatct ttgactactg ggggccaagga accctggtca ccgtctcctc ag

(12) Protein Sequence Defining Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region (without signal sequence)

(SEQ ID NO. 165) 1 evqlvqsgae vkkpgeslri sckgsgysft tywmhwvrqm pgkglewmge inptnghtny

61 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvss

(13) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region and Human IgG1 (Glm (17,1) allotype) Heavy Chain Constant Region (signal sequence underlined) (SEO ID NO. 166)

1 atggggtcaa ccgccateet cgcceteete etggetgtte tecaaggagt etgtgeegaa 61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaggatctcc 121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc 181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat 241 ccgtccttcc aaggccacgt caccatctca gctgacaagt ccatcagcac tgcctacctg 301 cagtggagca geetgaagge eteggacaee geeatgtatt aetgtgegag aaaetatgtt 361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc 421 aagggeeeat eggtetteee eetggeacee teeteeaaga geacetetgg gggeacageg 481 gccctqqqct qcctqqtcaa qqactacttc cccqaaccqq tqacqqtqtc qtqqaactca 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 601 teceteagea gegtggtgae egtgeeetee ageagettgg geaeceagae etacatetge 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 721 gacaaaactc acacatgeec acegtgeeca geacetgaac teetgggggg acegteagte 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtet ceaacaaage eeteecagee eecategaga aaaceatete caaageeaaa 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1261 gacggeteet tetteeteta cageaagete acegtggaca agageaggtg geageagggg 1321 aacgtettet catgeteegt gatgeatgag getetgeaca accaetaeae geagaagage 1381 ctctccctgt ctccgggtaa atga

(14) Protein Sequence Defining the Full Length Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (without signal sequence)

-continued (SEQ ID NO. 167) 1 evqlvqsgae vkkpgeslri sckgsgysft tywmhwvrqm pgkglewmge inptnghtny 61 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthccpc papellggps 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq 422 gnvfscsvmh ealhnhytqk slslspgk

(15) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region (signal sequence underlined) (SEO ID NO. 168)

<u>atgqqqtcaa ccqccatcct cqccctcct ctqqctqttc tccaaqqaqt ctqtqccqaa</u>
 gtqcaqctgg tgcaqtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc
 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
 ccqtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg
 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc ag

(16) Protein Sequence Defining Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Sequence (without signal sequence)

(SEQ ID NO. 169) 1 evqlvqsgae vkkpgeslki sckgsgysft tywmhwvrqm pgkglewmge inptnghtny

61 npsfqgqvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvss

(17) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region and Human IgG1 (Glm(17,1) allotype) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 170)

1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa 61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc 121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc 181 gggaaaggee tggagtggat gggggggatt aateetaeca aeggteatae taaetaeaat 241 ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg 301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt 361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc 421 aagggeecat eggtetteee eetggeacee teeteeaaga geacetetgg gggeacageg 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 601 teceteagea gegtggtgae egtgeeetee ageagettgg geaceeagae etacatetge 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 721 gacaaaactc acacatgece acegtgeeca geacetgaac teetgggggg acegteagte 781 tteetettee eeccaaaace caaggacace etcatgatet eeeggaceee tgaggteaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtet ceaacaaage eeteecagee eecategaga aaaceatete caaageeaaa

-continued

1081 gggcagecec gagaaceaca ggtgtacace etgececeat eeeggatga getgaceaag 1141 aaceaggtea geetgacetg eetggteaaa ggettetate eeagegacat egeeggagg 1201 tgggagagea atgggeagee ggagaacaae tacaagaeea egeeteeegt getggaetee 1261 gaeggeteet tetteeteta eageaagete acegtggaea agageagggt geageagggg 1321 aaegtettet eatgeteegt gatgeatgag getetgeaea aceaetaeae geagaagage 1381 eteteeetgt eteegggtaa atga

Two possible start ATGs are shown in uppercase.

Chain Variable Region (signal sequence underlined).

(SEO ID NO. 172) 1 ATGgacATGa gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc 61 <u>agatgtg</u>aca tecagatgae ceagteteea tecteetgt etgeatetgt aggagaeaga 121 gtcaccatca cttgcaaggc cagtgagaat gtggtttctt atgtatcctg gtatcagcag 181 aaaccaggga aagcccctaa gctcctgatc tatggggcat ccaaccggaa cactggggtc 241 ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg 301 caacetgaag attttgcaac ttactactgt gggcagagtt acaactatcc gtacacgttt 361 ggccagggga ccaagctgga gatcaaac (20) Protein Sequence Defining Humanized Hu2B8 Kv1-39.1 Kappa Chain Variable Region (without signal sequence) (SEO ID NO. 173) 1 diqmtqspss lsasvgdrvt itckasenvv syvswyqqkp gkapklliyg asnrntgvps 61 rfsgsgsgtd ftltisslqp edfatyycgq synypytfgq gtkleik (21) Nucleic Acid Sequence Encoding Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (SEQ ID NO. 174) 1 gaactgtggc tgcaccatct gtcttcatct tcccgccatc tgatgagcag ttgaaatctg 61 gaactgeete tgttgtgtge etgetgaata aettetatee eagagaggee aaagtaeagt 121 ggaaggtgga taacgccctc caatcgggta actcccagga gagtgtcaca gagcaggaca 181 gcaaggacag cacctacagc ctcagcagca ccctgacgct gagcaaagca gactacgaga

241 aacacaaagt ctacgcctgc gaagtcaccc atcagggcct gagctcgccc gtcacaaaga

301 gcttcaacag gggagagtgt tga

(19) Nucleic Acid Sequence Encoding Humanized Hu2B8 Kv1-39.1 Kappa

The first amino acid is derived from translation of the last nucleotide of variable region and beginning two nucleotides of the Kappa Light Chain sequence.

(22) Protein Sequence Defining Human Kappa Chain Constant Region $({\rm Km}\,(3)$ allotype) (allele 2).

(SEQ ID NO. 175) 1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd

61 skdstyslss tltlskadyc khkvyacevt hqglsspvtk sfnrgec

(23) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Kv1-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (signal sequence underlined)

(SEQ ID NO. 176) 1 <u>atgqacatqa qqqtccccqc tcaqctcctq qqqctcctqc tactctqqct ccqaqqtqcc</u> 61 <u>aqatqt</u>gaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga

121 gtcaccatca cttgcaaggc cagtgagaat gtggtttctt atgtatcctg gtatcagcag

181 aaaccaggga aagcccctaa gctcctgatc tatggggcat ccaaccggaa cactggggtc

241 ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg

301 caacctgaag attttgcaac ttactactgt gggcagagtt acaactatcc gtacacgttt

361 ggccagggga ccaagctgga gatcaaacga actgtggctg caccatctgt cttcatcttc

421 ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac

481 ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac

541 teccaggaga gtgtcacaga gcaggacage aaggacagea ectacageet cageageace

601 ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat

661 cagggeetga getegeeegt cacaaagage tteaacaggg gagagtgttg a

(24) Protein Sequence Defining the Full Length Humanized Hu2B8 Kv1-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 1)

(SEQ ID NO. 177) 1 diqmtqspss lsasvgdrvt itckasenvv syvswyqqkp gkapklliyg asnrntgvps

61 rfsgsgsgtd ftltisslqp edfatyycgq synypyttgq gtkleikrtv aapsvfifpp

121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt

181 lskadyekhk vyacevthqg lsspvtksfn rgec

(25) Nucleic Acid Sequence Encoding Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region (signal sequence underlined)

(SEQ ID NO. 178) 1 <u>atggaageee cagegeaget tetetteete etgetaetet ggeteeeaga taeeaetgga</u>

61 gaaatagtga tgacgcagte teeagecaee etgtetgtgt eteeagggga aagageeaee 121 eteteetgea aggeeagtga gaatgtggtt tettatgtat eetggtaeea geagaaaeet 181 ggeeaggete eeaggeteet eatetatggg geateeaaee ggaaeaetgg tateeeagee 241 aggtteagtg geagtgggte tgggaeagag tteaetetea eeateageag eetgeagtet

301 gaagattttg cagtttatta ctgtgggcag agttacaact atccgtacac gtttggccag

361 gggaccaagc tggagatcaa ac

(26) Protein Sequence Defining Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region (without signal sequence)

(SEQ ID NO. 179) 1 eivmtqspat lsvspgerat lsckasenvv syvswyqqkp gqaprlliyg asnrntgipa

61 rfsgsgsgte ftltisslqs edfavyycgq synypytfgq gtkleik

-continued

(27) Nucleic Acid Encoding the Full Length Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (signal sequence underlined) (SEQ ID NO. 180)

1 <u>atqqaaqccc caqcqcaqct tctcttcctc ctqctactct qqctcccaqa taccatqqa</u> 61 gaaatagtga tgacgcagtc tccagccacc ctgtctgtg ctccagggga aagagccacc 121 ctctcctgca aggccagtga gaatgtggtt tcttatgtat cctggtacca gcagaaacct 181 ggccaggetc ccaggetcct catctatggg gcatccaacc ggaacactgg tatcccagcc 241 aggttcagtg gcagtgggtc tgggacagag ttcactcta ccatcagcag cctgcagtct 301 gaagatttg cagtttatta ctgtgggcag agttacaact atccgtaca gtttggccag 361 gggaccaagc tggagatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 421 tctgatgagc agttgaaatc tggaactgc tctgttgtg gcctgctgaa taacttcat 481 cccagagagg ccaaagtaca gtggaagtg gataacgcc tccaatcggg taactccag 541 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 601 ctgagcaaag cagactacga gagettcaac aggggagagt gttga

(28) Protein Sequence Defining Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (without signal sequence)

(SEQ ID NO. 181) 1 eivmtqspat lsvspgerat lsckasenvv syvswyqqkp gqaprlliyg asnrntgipa

61 rfsgsgsgte ftltisslqs edfavyycgq synypytfgq gtkleikrtv aapsvfifpp

121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt

181 lskadyekhk vyacevthqg lsspvtksfn rgec

(2) Protein Sequence Defining Humanized LR2B8HC Heavy Chain Variable Region (without signal sequence)

(SEQ ID NO. 183) 1 qvqlvqpgae vvkpqtsvkl sckasgytft tywmhwvnqa pgqglewige inptnghtny

61 nekfkgkatl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvss

(3) Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1)

(SEO TD NO. 184) 1 ccagcacaaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg 61 gcacagegge eetgggetge etggteaagg actaetteee egaaceggtg aeggtgtegt 121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag 181 gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc acccagacct 241 acatetgeaa egtgaateae aageeeagea acaeeaaggt ggacaagaga gttgageeea 301 aatettgtga caaaacteac acatgteeac egtgeeeage acetgaacte etgggggggae 361 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc cggacccctg 421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt 481 acgtggacgg cgtggaggtg cataatgcca agacsaagcc gcgggaggag cagtacaaca 541 geacgtaceg tgtggtcage gteetcaceg teetgeacea ggactggetg aatggcaagg 601 agtacaagtg caaggtetee aacaaageee teecageeee categagaaa aceateteea 661 aagecaaagg geageeeega gaaceaeagg tgtacaeeet geeeeeatee egggaggaga 721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg 781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc 841 tggactccga cggctccttc ttcctctata gcaagctcac cgtggacaag agcaggtggc

76

-continued

901 agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc

961 agaagageet eteeetgtee eegggtaaat ga

For convenience, Table 13 provides a concordance chart showing the correspondence between the full length sequences and of the antibodies discussed in this section with those presented in the Sequence Listing.

75

chains. Variable region nucleic acid sequences were first synthesized by gene synthesis methods and then added to human constant region sequences. These human engineered antibodies were cloned into mammalian protein expression vectors,

TABLE 13

SEQ. ID. NO.	Protein or Nucleic Acid
154	Chimeric 2B8 IgG1 (G1m(17, 1)) – nucleic acid
155	Chimeric 2B8 IgG1 (G1m(17, 1)) – protein
156	Chimeric 2B8 Kappa (Km(3)) – nucleic acid
157	Chimeric 2B8 Kappa (Km(3)) – protein
158	Hu2B8 Hv1f.1 Heavy Chain Variable Region - nucleic acid
159	Hu2B8 Hv1f.1 Heavy Chain Variable Region - protein
160	Human IgG1 Heavy Chain Constant Region (G1m(17, 1)) allotype - nucleic acid
161	Human IgG1 Heavy Chain Constant Region (G1m(17, 1)) allotype - protein
162	Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17, 1) allotype) - nucleic acid
163	Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17, 1) allotype) - protein
164	Hu2B8 Hv5a.1 Heavy Chain Variable Region - nucleic acid
165	Hu2B8 Hv5a.1 Heavy Chain Variable Region – protein
166	Hu2B8 Hv5a.1 + IgG1 Constant (G1m(17, 1) allotype) - nucleic acid
167	Hu2B8 Hv5a.1 + IgG1 Constant (G1m(17, 1) allotype) - protein
168	Hu2B8 Hv5-51.1 Heavy Chain Variable Region - nucleic acid
169	Hu2B8 Hv5-51.1 Heavy Chain Variable Region - protein
170	Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17, 1 allotype) - nucleic acid
171	Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17, 1 allotype) - protein
172	Hu2B8 Kv1-39.1 Kappa Chain Variable Region – nucleic acid
173	Hu2B8 Kv1-39.1 Kappa Chain Variable Region - protein
174	Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) - nucleic acid
175	Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) - protein
176	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) - nucleic acid
177	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) - protein
178	Hu2B8 Kv3-15.1 Kappa Chain Variable Region - nucleic acid
179	Hu2B8 Kv3-15.1 Kappa Chain Variable Region – protein
180	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) - nucleic acid
181	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) – protein

B. Humanization Procedure 2

The second humanization method employed for reducing immunogenicity of the mouse 2B8 antibody is based on the method described in Studnicka et al. (1994) PROTEIN ENG. 45 7:805-814. The heavy and kappa human germline variable regions most identical (at the amino acid level) to those of mouse 2B8 were identified. Residues that differed between mouse and human were converted into the human sequence depending on the likely risk that such a change would affect 50 binding or immunogenicity. Low risk residues (i.e., residues that when changed would likely not affect antigen binding and would also reduce potential immunogenicity) were changed to the human amino acid in the heavy variable region (creating LR2B8HC) and the kappa variable region (creating 55 LR2B8LC). Additionally, low risk and medium risk (i.e., residues that when changed are somewhat likely to have an effect on antigen binding residues and would also reduce potential immunogenicity) were changed to the human amino acid in the heavy variable region (creating LRMR2B8HC) 60 and the kappa variable region (creating LRMR2B8LC). The human IgG1 heavy chain constant region (G1m(3) allotype (allele 1)) was added to the carboxyl terminus of the two human engineered heavy variable regions and the human Kappa constant region (Km(3) allotype (allele 1)) was added 65 to the carboxyl terminus of two human engineered light variable regions, thus creating four human engineered antibody

and protein was expressed in the four possible combinations of heavy chain plus light chain. Binding of the chimeric, chimeric/humanized, or humanized antibodies to human HGF was measured using conventional techniques, as described below.

The nucleic acid sequences encoding and the protein sequences defining each of the humanized antibodies are summarized below. In this section, the last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region junction. This nucleotide is included in the Variable Region because it is part of that exon. Amino acid sequences of Constant Regions listed below include the translation of this junction codon.

(1) Nucleic Acid Sequence Encoding the Humanized LR2B8HC Heavy Chain Variable Region (signal sequence underlined) (SEO ID NO. 182)

1 <u>atgggetggt catatattat tetetttett gttgetaceg</u> <u>ctacegatgt geactet</u>eaa

- 61 gtccaactcg tacaaccagg cgctgaagtc gtaaaacccg gaacatctgt taaactctca
- 121 tgcaaageet caggatacae tttcacaaet taetggatge attqqqtcaa teaageeece

10

15

20

-continued

- 181 ggacaaggcc tcgaatggat tggcgaaatt aacccaacta acggacatac taattataat
- 241 gaaaaattta agggcaaagc tacactcacc gtcgataaat caacctctac agcttatatg
- 301 gaactttcat ccctgagatc agaagataca gccgtctact attgcgccag aaactacgta
- 361 ggatcaatat tegattaetg gggteaagge acteteetea cagteagete ag

(2) Protein Sequence Defining Humanized LR2B8HC Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 183)

- l qvqlvqpgae vvkpgtsvkl sekasgytfi tywmhwvnqa pgqglewige inptnghtny
- 61 nekfkgkatl tvdkststay meissirsed tavyycarny vgsifdywgq gtlltvss

(3) Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region (Glm(3) allotype) (allele 1) (SEQ ID NO. 184)

- 1 ccageacaaa gggeceateg gtetteecee tggeaceete eteeaagage acetetgggg
- 61 gcacagegge ectgggetge etggteaagg actaetteee egaaceggtg aeggtgtegt
- 121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag
- 181 gactetacte ecteageage gtggtgaceg tgeeeteeag cagettggge acceagacet
- 241 acatetgeaa egtgaateae aageeeagea acaeeaaggt ggacaagaga gttgageeea

-continued

- 301 aatettgtga caaaaeteae acatgteeae egtgeeeage acetgaaete etgggggggae
- 361 cgtcagtett ectetteece ecaaaaeeea aggaeaeeet catgatetee eggaeeeetg
- 421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
- 481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
- 541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
- 601 agtacaagtg caaggtetee aacaaageee teecageeee categagaaa aceateteea
- 661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggaggaga
- 721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
- 781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc
- ²⁵ 841 tggactccga cggctccttc ttcctctata gcaagctcac cgtggacaag agcaggtggc
 - 901 agcaggggaa cgtettetea tgeteegtga tgeatgagge tetgeacaae caetaeaege
- 30 961 agaagageet eteeetgtee eegggtaaat ga

The first amino acid is derived from translation of the last nucleotide of variable region and the beginning two nucleotides of the IgG1 Heavy Chain sequence.

(4) Protein Sequence Defining Human IgG1 Heavy Chain Constant Region
 (Glm(3) allotype) (allele 1 or 2).
 (SEQ ID NO. 185)
 1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg

- 121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
- 181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
- 241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
- 301 qqgnvfscsv mhealhnhyt qkslslspgk

(5) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized LR2B8HC Heavy Chain Variable Region and Human IgGl Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (signal sequence underlined)

(SEQ ID NO. 186) 1 <u>atgggetggt catatattat tetettett gttgetaceg etacegatgt geaetet</u>caa 61 gteeaaeteg tacaaeeagg egetgaagte gtaaaaeeeg gaacatetgt taaaetetea 121 tgeaaageet eagaataea ttteaeaaet taetggatge attgggteaa teaageeeee 181 ggaeaaggee tegaatggat tggegaaatt aaeeeaaeta aeggaeatae taattataat 241 gaaaaattta agggeaaage taeaeteae gtegataaat eaaeetetae agettatatg 301 gaaettteat eeetgagate agaagataea geegtetaet attgegeeag aaaetaegta 361 ggateaatat tegattaetg gggteaagge acteteetea eagteagete ageeageaea 421 aagggeeeat eggtetteee eetggeaeee teeteeaaga geaeetetgg gggeaeageg

-continued 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 601 teectcagea gegtggtgae egtgeeetee ageagettgg geacecagae etacatetge 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt 721 gacaaaactc acacatgtcc accgtgccca gcacctgaac tcctgggggg accgtcagtc 781 tteetettee ceccaaaace caaggacace etcatgatet eceggaceee tgaggteaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtet ccaacaaage ceteccagee cccategaga aaaccatete caaagecaaa 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1261 gacggeteet tetteeteta tageaagete accgtggaea agageaggtg geageagggg 1321 aacgtettet catgeteegt gatgeatgag getetgeaca accaetaeae geagaagage 1381 ctctccctgt ccccgggtaa atga

(6) Protein Sequence Defining the Full Length Heavy Chain Humanized LR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (without signal sequence)

(SEQ ID NO. 187)
qvqlvqpae vvkpgtsvkl sckasgytft tywmhwvnqa pgqglewige inptnghtny
nekfkgkatl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvssas
tkgpsvfpla psskstsggt aalgclvkdy tpepvtvswn sgaltsgvht fpavlqssgl
yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps
vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt
knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq

(7) Nucleic Acid Sequence Encoding the Humanized LRMR2B8HC Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 188)

1 <u>atgggttggt catatattat actettete gtagecaceg ceaeegaegt acaetet</u>eag 61 gtteaaeteg taeaaecegg egeegaagte aagaaaecag gaacateagt eaaaetetea 121 tgtaaageaa geggataeae etttaetaet tattggatge attgggtaag acaageeeee 181 ggaeaaggae tegaatggat aggegaaata aateeeaeta atggaeatae aaattataat 241 eaaaaatte aaggaegege taeaeteaee gtegataaat eaaeeteaee egeataeatg 301 gaaeteaget eeeteegate gggaeaagga acaettetea eegtaagete ag

(8) Protein Sequence Defining Humanized LRMR2B8HC Heavy Chain Variable Region (without signal sequence)

(SEQ ID NO. 189) 1 qvqlvqpgae vkkpgtsvkl sckasgytft tywmhwvrqa pgqglewige inptnghtny

61 nqkfqgratl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvss

-continued

(9) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized LRMR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (signal sequence underlined)

(SEO ID NO. 190) 1 atgggttggt catatattat actetttete gtagecaceg ceaeegaegt acaeteteag 61 gttcaactcg tacaacccgg cgccgaagtc aagaaaccag gaacatcagt caaactctca 121 tgtaaagcaa gcggatacac ctttactact tattggatgc attgggtaag acaagccccc 181 ggacaaggac tcgaatggat aggcgaaata aatcccacta atggacatac aaattataat 241 caaaaatttc aaggacgcgc tacactcacc gtcgataaat caacctcaac cgcatacatg 301 gaactcagct ccctccgatc cgaagacact gccgtttatt attgtgccag aaactatgta 361 ggatctattt tcgattactg gggacaagga acacttctca ccgtaagctc agccagcaca 421 aagggeecat eggtetteee eetggeacee teeteeaaga geacetetgg gggeacageg 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 541 ggcgccctga ccagcggcgt gcacacette ccggctgtee tacagteete aggaetetae 601 teceteagea gegtggtgae egtgeeetee ageagettgg geaceeagae etacatetge 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt 721 gacaaaactc acacatgtcc accgtgccca gcacctgaac teetgggggg accgtcagte 781 tteetettee eeccaaaace caaggacace etcatgatet eeeggaceee tgaggteaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1261 gacggeteet tetteeteta tageaagete acegtggaea agageaggtg geageagggg 1321 aacgtettet catgeteegt gatgeatgag getetgeaca accaetaeae geagaagage 1381 ctctccctgt ccccgggtaa atga

(10) Protein Sequence Defining the Full Length Heavy Chain Humanized LRMR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (without signal sequence)

(SEQ ID NO. 191)

1 qvqlvqpgae vkkpgtsvkl sckasgytft tywmhwvrqa pgqglewige inptnghtny 61 nqkfqgratl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvssas 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq 421 gnvfscsvmh ealhnhytqk slslspgk

-continued

(11) Nucleic Acid Sequence Encoding the Humanized LR2B8LC Light Chain Variable Region (signal sequence underlined)

(SEQ ID NO. 192) 1 <u>atggaaagtc agaccettgt atteatetet attettettt ggttgtatgg ageagaegge</u>

61 gacattgtga tgacccaatc ccccgatagt atggccatga gtgtaggaga aagagtcacc

121 cttaattgca aagceteega aaatgtegtt teatatgtgt ettggtatea acaaaaaaece

181 ggccaatcac ccaaacttct catatacggc gcttcaaaca gaaacacagg cgttcccgac

241 agatttagtg gatccggatc agctacagat ttcaccctta ccatcagttc agttcaagca

301 gaagacgttg cagactatca ttgcggacaa tcttataact acccttacac attcggacaa

(12) Protein Sequence Defining Humanized LR2B8LC Light Chain Variable Region (without signal sequence)

(SEQ ID NO. 193) 1 divmtqspds mamsvgervt lnckasenvv syvswyqqkp gqspklliyg asnrntgvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleik

(13) Nucleic Acid Sequence Encoding the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1)

(SEQ ID NO. 194) 1 gtacggtggc tgcaccatct gtcttcatct tcccgccatc tgatgagcag ttgaaatctg

61 gaactgooto tgttgtgtgc ctgctgaata acttotatoo cagagaggoo aaagtacagt

121 ggaaggtgga taacgccctc caatcgggta actcccagga gagtgtcaca gagcaggaca

181 gcaaggacag cacctacagc ctcagcagca ccctgacgct gagcaaagca gactacgaga

241 aacacaaagt ctacgcctgc gaagtcaccc atcagggcct gagctcgccc gtcacaaaga

301 getteaacag gggagagtgt tag

The first amino acid is derived from translation of the last nucleotide of variable region and the beginning two nucle- ³⁵ otides of the Kappa Light Chain sequence.

(14) Protein Sequence Defining the Human Kappa Chain Constant Region $({\rm Km}\,(3)$ allotype) (allele 1).

(SEQ ID NO. 195) 1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd

61 skdstyslss tltlskadye khkvyacevt hqglsspvtk sfnrgec

(15) Nucleic Acid Sequence Encoding the Full Length Humanized LR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 196)

1 <u>atggaaaqte aqacettqt atteatete attettett gqttqtatqq aqeaqacqqe</u> 61 gacattgtga tgacceaate eccegatagt atggeeatga gtgtaggaga aagagteace 121 ettaattgea aageeteega aaatgtegtt teatatgtgt ettggtatea acaaaaaeee 181 ggeeaateae ecaaaettet eatataegge getteaaaea gaaaeaeagg egtteeagea 241 agatttagtg gateeggate agetaeagat tteaeeetta ecateagte agtteaagea 301 gaagaegttg eagaetatea ttgeggaeaa tettataaet aceettaa etteeggaeaa 361 ggaaceaaae tegaaattaa aegtaeggtg getgeaeeat etgetteat etteeegeea 421 tetgatgage agttgaaate tggaaetgee tetgttgtg geetgetgaa taaettetat 481 eeeagagagg eeaaagtaea gtggaaggtg gataaegeee teeaateggg taaeteeeag 541 gagagtgtea cagageagga eageaagaea ageaeetaea geeteagge eeateegg 601 etgageaaag eegeeteaga gagetteaae aggggagagt gttag

(SEO ID NO. 197)

(SEQ ID NO. 200)

-continued

(16) Protein Sequence Encoding the Full Length Humanized LR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1)

1 divmtqspds mamsvgervt lnckasenvv syvswyqkp gqspklliyg asnrntgvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleikrtv aapsvfifpp

121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt

181 lskadyekhk vyacevthqg lsspvtksfn rgec

(17) Nucleic Acid Sequence Encoding the Humanized LRMR2B8LC Light Chain Variable Region (signal sequence underlined)

(SEQ ID NO. 198) 1 <u>atggaateee aaaceettgt ttteatetet ateettetet ggetttatgg egeegaegga</u>

61 gacatcgtaa tgacacaatc ccctgactct cttgctatga gcttgggcga acgagtaaca

121 cttaactgca aagcatccga aaatgtcgta tcttacgtat cctggtatca gcaaaaacct

181 ggtcaaagtc ctaaacttct tatatatggt gcaagtaatc gtgaaagtgg cgtcccagac

241 agatttagcg gttcaggttc agcaactgac tttacactta caatttctag cgttcaggcc

301 gaagacgttg cagactatca ttgtggacaa tcttataact atccttatac tttcggacaa

361 ggcactaaac ttgaaattaa ac

(18) Protein Sequence Defining the Humanized LRMR2B8LC Light Chain Variable Region (without signal sequence)

(SEQ ID NO. 199) 1 divmtqspds lamslgervt lnckasenvv syvswyqqkp gqspklliyg asnresgvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleik

(19) Nucleic Acid Sequence Encoding the Full Length Humanized LRMR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (signal sequence underlined)

1 <u>atgqaatccc aaacccttqt tttcatctct atccttctt qqctttatqq cqccqacqqa</u>61 gacatcgtaa tgacacaatc ccctgactct cttgctatga gcttgggcga acgagtaaca121 cttaactgca aagcatccga aaatgtcgta tcttacgtat cctggtatca gcaaaaacct181 ggtcaaagtc ctaaacttct tatatatggt gcaagtaatc gtgaaagtgg cgtcccagac241 agatttagcg gttcaggttc agcaactgac tttacactta caattctag cgttcaggcc301 gaagacgttg cagactaca ttgtggacaa tcttataact atccttata cttccggacaa361 ggcactaaac ttgaaattaa acgtacggtg gctgcaccat ctgtcttcat cttcccgcca361 ggcactaaac ttgaaattaa acgtacggtg gctgcaccat ctgtcttcat cttcccgcca361 ggaagtgtc cagagtaca gtggaagtg gataacgccc tccaatcggg taacctccag361 cccaagagagg ccaaagtaca gtggaagtg gataacgccc tccaatcggg taaccccag361 ctgagcaag cagactacga gaacacaaa gtctacgcct gcgaagtca ccatcagggc661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag

(20) Protein Sequence Defining the Full Length Humanized LRMR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1)

(SEQ ID NO. 201) 1 divmtqapda lamalgervt lnckasenvv ayvawyqqkp gqapklliyg aanreagvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleikrtv aapsvfifpp

-continued

121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt

181 lskadyekhk vyacevthqg lsspvtksfn rgec

For convenience, Table 14 provides a concordance chart showing the correspondence between the full length

sequences and of the antibodies discussed in this section with those presented in the Sequence Listing.

TABLE 14

SEQ. ID NO.	Protein or Nucleic Acid
182	LR2B8HC Heavy Chain Variable Region – nucleic acid
183	LR2B8HC Heavy Chain Variable Region - protein
184	Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) – nucleic acid
185	Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) – protein
186	LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) – nucleic acid
187	LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) - protein
188	LRMR2B8HC Heavy Chain Variable Region - nucleic acid
189	LRMR2B8HC Heavy Chain Variable Region – protein
190	LRMR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) - nucleic acid
191	LRMR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) - protein
192	LR2B8LC Light Chain Variable Region – nucleic acid
193	LR2B8LC Light Chain Variable Region – protein
194	Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) - nucleic acid
195	Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) – protein
196	LR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – nucleic acid
197	LR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – protein
198	LRMR2B8LC Light Chain Variable Region – nucleic acid
199	LRMR2B8LC Light Chain Variable Region – protein
200	LRMR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – nucleic acid
201	LRMR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) - protein

Table 15 summarizes the heavy chain CDR sequences (Kabat Definition) of the humanized 2B8 antibodies prepared by humanization procedure 1 and by humanization procedure 2 described herein above in this Example.

Antibody	CDR1	CDR2	CDR3	Full Length Heavy Chain Variable Region
Murine 2B8 Heavy	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFKS (SEQ ID NO: 16)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 12
Hu2B8 Hv1f.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFQG (SEQ ID NO: 202)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 159
Hu2B8 Hv5a.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNPSFQG (SEQ ID NO: 203)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 165
Hu2B8 Hv5- 51.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNPSFQG (SEQ ID NO: 203)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 169
LR2B8HC	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFKG (SEQ ID NO: 204)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 183
LRMR2B8HC	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNQKFQG (SEQ ID NO: 205)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 189

TABLE 15

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Table 16 summarizes the light chain CDR sequences (Kabat Definition) of the humanized 2B8 antibodies prepared by humanization procedure 1 and by humanization procedure 2 described herein above in this Example.

TABLE 16

Antibody	CDR1	CDR2	CDR3	Full Length Light Chain Variable Region
Murine 2B8 Light	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 14
Hu2B8 Kv1-39.1	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 173
Hu2B8 Kv3-15.1	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 179
LR2B8LC	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 193
LRMR2B8LC	KASENVVSYVS (SEQ ID NO: 18)	GASNRES (SEQ ID NO: 206)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 199

C. Binding Affinity of Humanized 2B8 Antibodies

Antigen-binding affinity and kinetics of interaction were assessed by surface plasmon resonance technology using a BIAcore T100 instrument. Mouse anti-human immunoglobulins (Jackson ImmunoResearch Labs, 209-005-098) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1005-34) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's recommendations. The analyses were performed at 25° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfac-40 tant P20 (BIAcore, Catalog No. BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer.

The antibodies were captured on individual flow cell at a flow rate of 10 μ L/min. Injection time was variable for each 45 antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at $_{50}$ $60\,\mu$ L/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10 mM Glycine-HCl, pH 2.0 (BIAcore, Catalog No. BR-1003-55) injected for 3 minutes at a flow rate of 60 µL/min before another cycle was initiated. HGF con- 55 centrations tested were 1.88, 3.75 and 7.5 nM. Determination of kinetic parameters was achieved using the kinetic function of the BIAevalutation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_{d} (dissociation rate constant) and K_{D} (equilibrium ₆₀ dissociation constant) are summarized in FIG. 8.

The results summarized in FIG. 8 show that certain combinations of superhumanized heavy chains (Hu2B8 Hv5a.1, Hu2B8 Hv5-51.1 or Hu2B8 Hv1-f.1) and light chains (Hu2B8 Kv1-39.1 or Hu2B8 Kv3-15.1) retain similar bind-65 ing affinity (K_D) to HGF as chimeric 2B8 (mouse variable regions with human constant regions) and 2B8 (Table 5).

D. Mutually Exclusive Binding Assay

Mutually exclusive binding to HGF was assessed by surface plasmon resonance technology using a BIAcore T100 instrument. Mouse anti-human immunoglobulins (Jackson ImmunoResearch Labs, 209-005-098) were immobilized on carboxymethylated dextran CM5 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's recommendations. The analyses were performed at 25° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, #BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/ml CM-Dextran Sodium salt (Fluka, Catalog No. 15 86524) as running buffer.

The humanized antibodies were captured on an individual flow cell at a flow rate of 30 µL/min. Injection time was variable for each antibody to yield approximately 150 RU of antibody captured for each cycle. HGF (R&D Systems, Cata-20 log No. 294-HGN-025) diluted in running buffer at a final concentration of 7.5 µg/mL was injected for 90 sec at 30 µL/min over the captured humanized antibodies. Binding of HGF was monitored before subsequent injection of mouse 2B8 antibody or polyclonal goat anti-HGF antibody (R & D Systems, AF294) for 3 min at 30 µL/min. The surface then was regenerated with 10 mM Glycine-HCl, pH 2.0 (BIAcore, Catalog No. BR-1003-55) injected for 3 min at a flow rate of 60 µL/min before another antibody was tested. The results are summarized in FIG. 9.

Results summarized in FIG. 9 show that both humanized 2B8 antibodies and chimeric 2B8 antibodies prevent murine 2B8 from binding HGF. These results demonstrate that the humanized antibodies still bind the same HGF epitope as the 35 original 2B8 antibody.

Example 13

Production of Humanized 2B8 Variants

a. HUMAN ENGINEEREDTM Antibodies

Codon- and expression-optimized low risk and low-plusmoderate risk Human Engineered light chain (LR2B8LC and LRMR2B8LC, respectively) and heavy chains (LR2B8HC and LRMR2B8HC, respectively) were cloned in-phase into XOMA's transient antibody expression vectors, which contain human Kappa and Gamma-1 constant regions modules. The four Human Engineered 2B8 variants were produced by transient transfection in HEK293E cells. The following four antibodies were produced:

- HE2B8-1=LR2B8HC (+IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 187) plus LR2B8LC (+Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)
- HE2B8-2=LR2B8HC (+IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 187) plus LRMR2B8LC (+Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 201)
- HE2B8-3=LRMR2B8HC (+IgG1 constant region (G1m (3) allotype (allele 1)) (SEQ ID NO. 191) plus LR2B8LC (+Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)
- HE2B8-4=LRMR2B8HC (+IgG1 constant region (G1m (3) allotype (allele 1)) (SEQ ID NO. 191) plus LRMR2B8LC (+Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 201)

The light and heavy chains were co-transfected into XOMA's suspension adapted HEK293E cells grown in IS293 media (Irvine Scientific, Irvine, Calif.) using 2 liter shake flasks. After 24 hours in the shake flasks, 200 mL of transfected cells were centrifuged, resuspended in 40 mL of ⁵ fresh medium and transferred to Integra flasks (Wilson Wolf Manufacturing Inc., MN) for production. After incubation for seven days, the cell suspensions were removed from the Integra flasks, centrifuged and the culture supernatants retained. ¹⁰ Antibodies in the culture supernatants were purified on protein A spin columns (Pro-Chem), dialyzed against PBS, concentrated and sterile filtered.

b. SUPERHUMANIZED[™] Antibodies

15 Full length Hu2B8_Hv5-51.1+human IgG1 constant domain (G1 m(3) allotype) cDNA was cloned into pEE6.4 (Lonza Biologics, Berkshire, UK) using HindIII and EcoRI restriction sites. Full length Hu2B8_Kv1-39.1 variable 20 region+human Kappa constant domain cDNA and full length Hu2B8_Kv3-15.1 variable region+human Kappa constant domain cDNA were each cloned into pEE14.4 (Lonza Biologics) using HindIII and EcoRI restriction sites. The hCMV-MIE promoter+full length Hu2B8_Hv5-51.1+human IgG1 $^{\ 25}$ constant domain (G1m(3) allotype) cDNA+SV40 poly A fragment (in pEE6.4) was removed by NotI/SalI digestion and inserted into either Kappa chain pEE14.4 vector through NotI/SalI sites, thus creating 2 different expression vectors that each simultaneously express heavy and light chain to make the following antibodies:

sh2B8-9 (G1m(3))=hu2B8 Hv5-51.1 (+IgG1 constant region (G1m(3) allotype) (allele 2)) (SEQ ID NO. 210) ³⁵ plus hu2B8 Kv 1-39.1 (+Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO: 177)

sh2B8-12 (G1m(3))=hu2B8 Hv5-51.1 (+IgG1 constant region (G1m(3) allotype) (allele 2)) (SEQ ID NO. 210) 40 plus hu2B8 Kv 3-15.1 (+Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID No. 181)

The nucleic acid sequences encoding and the protein sequences defining the human IgG1 Heavy Constant Region G1m(3) allotype (allele 2) and each of the full length heavy ⁴⁵ chain sequences are set forth below. The light chain sequences were the same as described in Example 12.

(1) Nucleic Acid Sequence Encoding Human IgG1 Heavy Chain Constant Region (Glm(3) allotype) (allele 2) (SEQ ID NO. 207)

- 1 cetecaceaa gggeecateg gtetteeeee tggeaceete etecaagage acetetgggg
- 61 gcacagegge eetgggetge etggteaagg actaetteee egaaceggtg aeggtgtegt
- 121 ggaactcagg cgccctgacc agcggcgtgc acacetteec ggctgteeta cagteeteag
- 181 gactetacte ceteageage gtggtgaeeg tgeeeteeag cagettggge acceagaeet
- 241 acatetgeaa egtgaateae aageeeagea acaeeaaggt ggacaagaga gttgageeea
- 301 aatottgtga caaaactcac acatgcoccac cgtgcoccagc acotgaactc ctgggggggac
- 361 cgtcagtett cetetteece ecaaaaceea aggacaeeet catgatetee eggaceeetg
- 421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
- 481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
- 541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
- 601 agtacaagtg caaggtetee aacaaageee teecageeee categagaag accateteea
- 661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggaggaga
- 721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
- 781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctccgtgc
- 841 tggacteega eggeteette tteetetaea geaageteae egtggacaag ageaggtgge
- 901 agcaggggaa cgtettetea tgeteegtga tgeatgagge tetgeacaae caetaeaege
- 961 agaagageet eteeetgtet eegggtaaat ga
- ² The first amino acid is derived from translation of the last nucleotide of variable region and the beginning two nucleotides of the IgG1 Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss

61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg

121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreegyn

181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree

241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw

301 qqgnvfscsv mhealhnhyt qkslslspgk

(3) Nucleic Acid Sequence Encoding the Full Length Chain Containing Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region and the Human IgG1 Heavy Chain Constant Region Glm(3) allotype (allele 2) (signal sequence underlined)

-continued

(SEO ID NO. 209) 1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa 61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc 121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc 181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat 241 ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg 301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt 361 ggtagcatet ttgactaetg gggecaagga accetggtea cegteteete ageeteeace 421 aaqqqcccat cqqtcttccc cctqqcaccc tcctccaaqa qcacctctqq qqqcacaqcq 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 601 teceteagea gegtggtgae egtgeeetee ageagettgg geaceeagae etacatetge 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt 721 gacaaaactc acacatgccc accgtgccca gcacctgaac teetgggggg accgtcagtc 781 tteetettee eeccaaaace caaggacace etcatgatet eeeggaceee tgaggteaca 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1021 tgcaaggtet ccaacaaage ceteccagee cccategaga agaceatete caaageeaaa 1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1261 gacggeteet tetteeteta cagcaagete accgtggaca agagcaggtg geageagggg 1321 aacgtettet catgeteegt gatgeatgag getetgeaca aceaetaeae geagaagage 1381 ctctccctgt ctccgggtaa atga (4) Protein Sequence Defining the Full Length Heavy Chain Containing Humanized Hu2B8 Hv5-51.1 and the Human IgG1 Heavy Chain Constant Region G1m(3) allotype (allele 2) (without signal sequence) (SEQ ID NO. 210) 1 evqlvqsgae vkkpgeslki sckgsgysft tywmhwvrqm pgkglewmge inptnghtny 61 npsfqgqvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt

361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq

94

Each dual expression vector was transfected into 293T cells for transient expression using DMEM 10% fetal bovine serum. Forty-eight hours after transfection, cells were washed with and then replaced with serum free medium, IS GROTM (Irvine Scientific, Santa Ana, Calif.) containing 4 5 mM L-Glutamine. Supernatant was harvested daily and replaced with fresh media for 10 days. The culture supernatants were centrifuged, filtered (0.45 µm) and concentrated 10-100 fold. Antibodies were purified on ProSep vA resin (Millipore), dialyzed against PBS, concentrated and sterile 10 filtered.

Example 14

Binding Characteristics of Humanized 2B8 Variants

The humanized antibodies produced in Example 13 were characterized by their ability to bind hHGF and the recombinant HGF proteins produced in Example 3.

The antibodies were analyzed by surface-plasmon reso- 20 nance using a BIAcore T100 instrument to assess their ability to bind hHGF and the fusion proteins discussed in Example 3. Each antibody was immobilized on a carboxymethylated dextran CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) 25 using a standard coupling protocol according to manufacturer's instructions.

Analyses were performed at 25° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. R-1000-54), 2 mg/mL BSA (EMD, 30 Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer. Supernatant containing different HGF fusion proteins or supernatant from cells transfected with empty vector were injected over each antibody at a flow rate of 30 µL/min for 3 minutes. The 35 resulting binding was determined as resonance units (RU) over baseline 30 seconds after the end of injection. Binding was compared to human HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer. Non-specific binding was monitored by comparing binding to a control surface. The results are summarized in the Table 17.

Mouse anti-human immunoglobulins (Jackson Labs, Catalog No. 209-005) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. The analyses were performed at 25°C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. BR-1000-54), and 2 mg/mL BSA (EMD, Catalog No. 2930).

The antibodies were captured in an individual flow cell at a flow rate of 10 μ L/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at 60 µL/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10 mM Glycine-HCl, pH 2.2 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 µL/min before another cycle was initiated. HGF concentrations tested were 0.46 nM to 7.5 nM.

Kinetic parameters were determined using the kinetic function of the BIAevalutationTM software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized in Table 18.

TABLE 18

Antibody	$\mathbf{k}_{a}\left(1/\mathrm{Ms}\right)$	$\mathbf{k}_{d}\left(1/\mathbf{s}\right)$	$K_D(pM)$	$^{\mathrm{SD}}$
2B8	1.4×10^6	1.0×10^{-5}	7.3	_
HE2B8-1	2.2×10^{6}	1.4×10^{-5}	7.1	5.2
HE2B8-2	1.8×10^{6}	9.6×10^{-6}	5.2	2.7
HE2B8-3	2.0×10^{6}	4.1×10^{-6}	2.0	1.1
HE2B8-4	1.7×10^{6}	1.1×10^{-5}	6.5	1.3
sh2B8-9 (G1m(17, 1)	2.0×10^{6}	1.7×10^{-5}	8.1	5.3
sh2B8-12(G1m(17, 1))	1.9×10^{6}	2.3×10^{-5}	12	0.4

TA T	$\mathbf{D} \mathbf{L} \mathbf{D}$	17
\mathbf{IA}	DLE	-17

Antibody	rhHGF (R&D Systems)	rmHGF (R&D Systems)	MHM chimera (495-585)	MHM chimera (507-585)	MHM chimera (499-556)
2B8	Yes	No	Yes	Yes	Yes
HE2B8-1	Yes	No	Yes	Yes	Yes
HE2B8-2	Yes	No	Yes	Yes	Yes
HE2B8-3	Yes	No	Yes	Yes	Yes
HE2B8-4	Yes	No	Yes	Yes	Yes
sh2B8-9	Yes	No	Yes	Yes	Yes
(G1m(3))					
sh2B8-12 (G1m(3))	Yes	No	Yes	Yes	Yes

The results in Table 17 demonstrate that each of the humanized 2B8-based antibodies bind rhHGF and all three mousehuman-mouse chimeras.

Example 15

Binding Affinities of Humanized 2B8 Variants

The binding affinities and kinetics of interaction of the 65 antibodies listed in Table 15 were measured by surface plasmon resonance.

These data show that the humanized antibodies have fast association rates (k_a) , very slow dissociation rates (k_d) , and very high affinities (K_D) . In particular, the antibodies have affinities ranging from 2.0-12 pM.

Example 16 Comparison of Binding Affinities at 25° C. and 37°

The binding affinities and kinetics of interaction of antibody HE2B8-4, sh2B8-9, sh2B8-12, and murine 2B8 were measured by surface plasmon resonance under different conditions.

55

60

43

50

55

60

 $K_D(pM)$

13.5

4.5

5.6

3.3

8.1

5.8

12.0

4.8

 2.1×10^{-5}

 1.3×10^{-5}

 1.2×10^{-5}

 1.0×10^{-5}

 1.7×10^{-5}

 1.4×10^{-5}

 2.3×10^{-4}

 1.1×10^{-5}

Mouse anti-human immunoglobulins (Jackson Labs, Catalog No. 209-005) or rabbit anti-mouse immunoglobulins (BIAcore, Catalog No. BR-1005-14) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. In the case of 25° C. measurements for sh2b8-9 and sh2B8-12, a CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) was used. The analyses were performed at 25° C. and 37° C. using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. BR-1000-54), and 2 mg/mL BSA (EMD, Catalog No. 2930) as running buffer.

The antibodies were captured in an individual flow cell at a flow rate of 10 µL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected 20 sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at $60\,\mu$ L/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface of mouse 25 anti-human immunoglobulins sensor chips was then regenerated with 10 mM Glycine-HCl, pH 2.2 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 µL/min before another cycle was initiated. The surface of rabbit anti-mouse immunoglobulins sensor chips was regen- 30 erated with 10 mM Glycine-HCl, pH 1.7 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 µL/min before another cycle was initiated. HGF concentrations tested were 0.46 nM to 7.5 nM.

Kinetic parameters were determined using the kinetic ³⁵ function of the BIAevaluation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized below in Table 40 19.

it), \mathbf{k}_d (dissociation constant)	ation rate ant) are sur	constant) a nmarized b
T	ABLE 19	
Temp. (° C.)	$\mathbf{k}_a(1/\mathrm{Ms})$	$\mathbf{k}_{d}\left(1/\mathbf{s}\right)$

 1.6×10^{6}

 2.8×10^6

 2.0×10^{6}

 3.1×10^{6}

 2.0×10^6

 2.5×10^{6}

 1.9×10^{6}

 2.4×10^{6}

25

37

25

37

25

37

25

37

Antibody

HE2B8-4

HE2B8-4

sh2B8-9

sh2B8-9

(G1m(3))

sh2B8-12

sh2B8-12

(G1m(3))

(G1m(17, 1))

(G1m(17, 1))

2B8

2B8

As expected, the association rate constants increased with an increase in the temperature. Surprisingly, the dissociation ₆₅ constants did not change significantly with a corresponding increase in temperature. Consequently, the overall equilib-

rium dissociation constants (K_D) were approximately 1.4 to 3 times smaller (higher affinity) at physiological temperature (37° C.).

Example 17

Neutralization Activity of Humanized 2B8 Variants

The antibodies described in Example 14 were characterized for their ability to (a) inhibit the binding of hHGF to c-Met, and (b) inhibit HGF stimulated BrdU incorporation in 4 MBr-5 cells.

HGF-Met Binding Inhibition Assay (Neutralization Assay) was performed as described in as follows. The antibodies were tested by ELISA for their ability to inhibit hHGF binding to c-Met. Specifically, Wallac 96-well DELFIA assay plates (Wallac Inc., Catalog No. AAAND-0001) were coated with 100 µL of 6.25 µg/mL HGF (R&D Systems, Catalog No. 294-HGN-025) in carbonate coating buffer (15 mM Na₂CO₃ and 34 mM NaHCO₃, pH 9.0) for 16 hours at 4° C. The plates then were blocked with 200 µL of 5% non-fat dry milk in PBS for 1 hour at room temperature. The antibodies were prepared in a separate plate by adding increasing concentrations of the antibodies under investigation (0.033-250 nM, 2-fold-serial dilution) to 2 nM biotinylated c-Met in 5% non-fat dry milk in PBS. c-Met (R&D Systems, Catalog No. 358-MT/CF) is biotinylated according to manufacturer's instruction at 10:1 biotin to c-Met ratio (Pierce, Catalog No. 21335). 100 µL of sample per well was transferred to the assay plate and incubated for 2 hours at room temperature. The resulting plates were washed three times with PBS-0.1% Tween 20, and incubated for 1 hour at room temperature with Eu-labeled Streptavidin (Wallac, Catalog No. 1244-360) diluted 1:1000 in DELFIA assay buffer (Wallac, Catalog No. 4002-0010). The resulting plates were washed 3 times with DELFIA wash solution (Wallac, Catalog No. 4010-0010) and incubated with 100 µL/well DELFIA enhancement solution (Wallac #4001-0010) for 15 minutes at room temperature with agitation. The plates were read on Victor³V instrument (Perkin Elmer) using the Europium method. The IC50 values were calculated using Prism.

The IC_{50} values obtained are shown in Table 20.

TABLE 20

_			
	Antibody	$IC_{50}\left(nM\right)$	$^{\rm SD}$
	2B8	9.2	1.2
	HE2B8-1	6.0	1.2
	HE2B8-2	5.7	1.1
	HE2B8-3	5.9	1.1
	HE2B8-4	6.5	1.2
	sh2B8-9 (G1m(3))	4.2	_
	sh2B8-12 (G1m(3)	6.8	

These results from Table 20 demonstrate that the humanized antibodies tested efficiently neutralize HGF binding to c-Met.

The antibodies in Table 17 were also tested in the cell proliferation assay described in Example 7(b). The results are summarized below in Table 21.

TABLE 21

Antibody	$\mathrm{IC}_{50}\left(nM\right)$	SD	
2B8	0.86	0.35	
HE2B8-1	0.47	0.15	

TABLE 21-continued

Antibody	$IC_{50}\left(nM\right)$	SD	
HE2B8-2	0.66	0.13	
HE2B8-3	0.55	0.28	
HE2B8-4	0.58	0.26	
sh2B8-9 (G1m(3))	0.52	0.11	
sh2B8-12 (G1m(3))	0.81	0.22	1

The results from Table 21 demonstrate that all the humanized antibodies tested inhibit HGF-induced proliferation of 4 MBr-5 cells.

Example 18

Anti-Scatter Activity of Humanized 2B8 Variants

The antibodies in Table 17 were tested in the anti-scatter assay described in Example 8. The results are summarized below in Table 22.

TABLE ZZ	ΤA	BI	Æ	22
----------	----	----	---	----

Inhibition of HC	F-induced MDCK Cell	_	
Antibody	Trial 1	Trial 2	3
2B8	++	++	
HE2B8-1	++	++	
HE2B8-2	++	++	
HE2B8-3	++	++	
HE2B8-4	++	++	
sh2B8-9 (G1m(3))	++	++	
sh2B8-12 (G1m(3))	++	++	

- No Inhibition

+++ Very strong, nearly complete inhibition

++ Strong inhibition

+ Detectable inhibition

The results in Table 22 demonstrate that all the humanized antibodies tested inhibited HGF-induced scattering to the same extent as the murine monoclonal antibody 2B8.

Example 19

Inhibition of HGF-Stimulated c-Met Phosphorylation

The antibodies in Table 17 were tested in the c-Met phosphorylation assay described in Example 9. The results are summarized below in Table 23.

		_	
TA	DI	LZ -	72
1/3	1.21	11	23

Antibody	Average of Two Trials	Standard Deviation	
2B8	0.91	0.02	
he2B8-1	0.80	0.04	
he2B8-2	0.88	0.15	
he2B8-3	0.79	0.05	
he2B8-4	0.75	0.14	
sh2B8-9 (G1m(3))	0.93	0.03	
sh2B8-12 (G1m(3))	0.81	0.07	

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The results in Table 23 demonstrate that all the humanized antibodies tested are potent inhibitors of HGF-induced c-Met phosphorylation in PC-3 cells.

Example 20

Tumor Inhibition in U87MG Xenograft Model

The ability of the humanized monoclonal antibodies of the invention to inhibit tumor growth was tested in an U87MG xenograft model. U87MG cells (ATCC) were expanded in culture at 37° C. in an atmosphere containing 5% CO₂ and 95% air, using a medium comprising Dulbecco's Modified Eagle medium (DMEM) with 10% fetal bovine serum, 100 units/mL penicillin and 100 μ g/mL streptomycin. The cells were subcultured and maintained by detaching the cells from the wall of the culture dish using trypsin-EDTA.

Near-confluent cells were collected by trypsinization and then 5×10^6 cells in 50% Matrigel (BD Biosciences; catalog no. 356237) were injected subcutaneously into the upper dorsal area between the shoulder blades of 7-week old female ICR SCID mice (Taconic Labs). The long (L) and short (W) diameters (mm) of tumors were measured with a caliper. Tumor volume (vol.) was calculated as: volume (mm³)=L× $W^2/2$. When the tumors grew to approximately 200 mm³, the 25 tumor-bearing mice were randomized into 5 groups of 10 mice each. One group received PBS and one group received human IgG control. Each of the other 4 groups received one of the humanized antibodies (HE2B8-1, HE2B8-2, HE2B8-3, and HE2B8-4). All the antibodies were dosed at 0.25 mg/kg body weight, twice per week, by intra-peritoneal injections of 5 doses. Tumor volumes and mouse body weights were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test.

The humanized antibodies tested were active in vivo. There was 57% tumor growth inhibition for HE2B8-1 with a p value of 0.02, 61% tumor growth inhibition for HE2B8-2 with a p value of 0.02, 85% tumor growth inhibition for HE2B8-3, with a p value of 0.0004, and 74% tumor growth inhibition for HE2B8-4 with a p value of 0.001. No significant body weight loss was observed.

A subsequent study was performed as described above in female NCR nude mice (Taconic Labs) bearing subcutaneous U87MG tumors inoculated in the flank. Each group (10 mice each) received one of the following treatments at 0.5 mg/kg: PBS vehicle control, huIgG control, HE2B8-4, or sh2B8-9. Treatment was given intra-peritoneal twice weekly for a minimum of 5 weeks. Each treatment group demonstrated similar tumor regression with tumor growth inhibition of 113% for sh2B8-9 and 115% for HE2B8-4, and a minimum tumor growth delay of 30 days. Both treatments were well-tolerated with no significant body weight loss.

INCORPORATION BY REFERENCE

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

EQUIVALENTS

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

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360

385
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60

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240

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360

420

424

132

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142

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ggaagattga tggcagtgaa cgacaaaatg gcgtcctgaa cagttggact gatcaggaca	180										
gcaaagacag cacctacagc atgagcagca ccctcacgtt gaccaaggac gagtatgaac	240										
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ggaagattga tggcagtgaa cgacaaaatg gcgtcctgaa cagttggact gatcaggaca	180
gcaaagacag cacctacagc atgagcagca ccctcatgtt gaccaaggac gagtatgaac	240
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Phe Val	. Phe 35	Asp	ГЛа	Ala	Arg	Lys 40	Gln	Суз	Leu	Trp	Phe 45	Pro	Phe	Asn
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Pro Trp) Ser	Ser 100	Met	Ile	Pro	His	Glu 105	His	Ser	Phe	Leu	Pro 110	Ser	Ser
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Pro Glu	ı Arg 195	Tyr	Pro	Asp	Гла	Gly 200	Phe	Asp	Asp	Asn	Tyr 205	Cys	Arg	Asn
Pro Asp 210) Gly	Gln	Pro	Arg	Pro 215	Trp	Cys	Tyr	Thr	Leu 220	Asp	Pro	His	Thr
Arg Tr <u>p</u> 225	Glu	Tyr	Сүз	Ala 230	Ile	Гла	Thr	Суз	Ala 235	Asp	Asn	Thr	Met	Asn 240
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Gly Tyr	с Сув	Ser	Gln 325	Ile	Pro	Asn	Суз	Asp 330	Met	Ser	His	Gly	Gln 335	Aab
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His Arc 370	g His)	Ile	Phe	Trp	Glu 375	Pro	Asp	Ala	Ser	Lуз 380	Leu	Asn	Glu	Asn
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Pro	Ala	Pro	Ile	Glu 805	Lys	Thr	Ile	Ser	Lys 810	Ala	Lys	Gly	Gln	Pro 815	Arg

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Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys 820 825 830 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp 835 840 845 Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys 850 855 860 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser 865 870 875 880 Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser 885 890 895 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser 900 905 910 Leu Ser Leu Ser Pro Gly Lys 915 <210> SEQ ID NO 120 <211> LENGTH: 2901 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Synthetic mhm (V495-L585)-Fc chimeric protein <400> SEQUENCE: 120 atgatgtggg ggaccaaact tctgccggtc ctgttgctgc agcatgtcct cctgcacctc 60 ctcctgcttc atgtcgccat cccctatgca gaaggacaga agaaaagaag aaatacactt 120 catgaattta aaaagtcagc aaaaactact cttaccaagg aagacccatt actgaagatt 180 aaaaccaaaa aagtgaactc tgcagatgag tgtgccaaca ggtgtatcag gaacaggggc 240 tttacgttca cttgcaaggc cttcgttttt gataagtcaa gaaaacgatg ctactggtat 300 cctttcaata gtatgtcaag tggagtgaaa aaagggtttg gccatgaatt tgacctctat 360 gaaaacaaag actatattag aaactgcatc attggtaaag gaggcagcta taaagggacg 420 gtatccatca ctaagagtgg catcaaatgc cagccttgga attccatgat cccccatgaa 480 cacagetate geggtaaaga eetaeaggaa aactaetgte gaaateeteg aggggaagaa 540 gggggaccct ggtgtttcac aagcaatcca gaggtacgct acgaagtctg tgacattcct600 cagtgttcag aagttgaatg catgacctgc aatggtgaaa gctacagagg tcccatggat 660 cacacagaat caggcaagac ttgtcagcgc tgggaccagc agacaccaca ccggcacaag 720 ttcttgccag aaagatatcc cgacaagggc tttgatgata attattgccg caatcctgat 780 ggcaagccga ggccatggtg ctacactctt gaccctgaca ccccttggga gtattgtgca 840 attaaaacgt gegeteacag tgetgtgaat gagaetgatg teeetatgga aacaactgaa 900 tgcattcaag gccaaggaga aggttacagg ggaaccagca ataccatttg gaatggaatt 960 ccctgtcagc gttgggattc gcagtaccct cacaagcatg atatcactcc cgagaacttc 1020 1080 aaatgcaagg accttagaga aaattattgc cgcaatccag atggggctga atcaccatgg 1140 tgttttacca ctgacccaaa catccgagtt ggctactgct ctcaaattcc caagtgtgac gtgtcaagtg gacaagattg ttatcgtggc aatgggaaaa attacatggg caacttatcc 1200 aaaacaaggt ctggacttac atgttccatg tgggacaaga atatggagga tttacaccgt 1260 catatettet gggagecaga tgetageaaa ttgaataaga attaetgeeg gaateetgat 1320 gatgatgccc atggaccttg gtgctacacg gggaatcctc ttattccttg ggattattgc 1380 cctatttccc gttgtgaagg agatactaca cctacaattg tcaatttgga ccatcctgta 1440

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Phe Val Phe 35	Asp Lys Se	er Arg Lys 1 40	Arg Cys Tyr	Trp Tyr Prc 45	Phe Asn							
Ser Met Ser 50	Ser Gly Va	al Lys Lys (55	Gly Phe Gly	His Glu Phe 60	e Asp Leu							
Tyr Glu Asn 65	Lys Asp Ty 7(yr Ile Arg 2)	Asn Cys Ile 75	Ile Gly Lys	Gly Gly 80							
Ser Tyr Lys	Gly Thr Va 85	al Ser Ile '	Thr Lys Ser 90	Gly Ile Lys	95 Cys Gln							
Pro Trp Asn	Ser Met I]	le Pro His (Glu His Ser	Tyr Arg Gly	' Lys Asp							

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Ala 225	Ile	Lys	Thr	Суз	Ala 230	His	Ser	Ala	Val	Asn 235	Glu	Thr	Asp	Val	Pro 240
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Gln	Tyr	Pro 275	His	Lys	His	Asp	Ile 280	Thr	Pro	Glu	Asn	Phe 285	Lys	Суз	Гла
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Trp 305	Cys	Phe	Thr	Thr	Asp 310	Pro	Asn	Ile	Arg	Val 315	Gly	Tyr	Суз	Ser	Gln 320
Ile	Pro	Lys	Суз	Asp 325	Val	Ser	Ser	Gly	Gln 330	Asp	Сүз	Tyr	Arg	Gly 335	Asn
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Ile 465	Lys	Glu	Ser	Trp	Val 470	Leu	Thr	Ala	Arg	Gln 475	Сүз	Phe	Pro	Ser	Arg 480
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Arg	Gly	Asp	Glu 500	Lys	Cys	Lys	Gln	Val 505	Leu	Asn	Val	Ser	Gln 510	Leu	Val
Tyr	Gly	Pro 515	Glu	Gly	Ser	Asp	Leu 520	Val	Leu	Met	Lys	Leu 525	Ala	Arg	Pro

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Pro	Cys 610	Glu	Gly	Asp	Tyr	Gly 615	Gly	Pro	Leu	Ile	Cys 620	Glu	Gln	His	Lys
Met 625	Arg	Met	Val	Leu	Gly 630	Val	Ile	Val	Pro	Gly 635	Arg	Gly	Сүз	Ala	Ile 640
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Ile	His	Lys	Val 660	Ile	Leu	Thr	Tyr	Lys 665	Leu	Cys	Gly	Arg	His 670	His	His
His	His	His 675	Ser	Ala	Gly	Leu	Val 680	Pro	Arg	Gly	Ser	Asp 685	Lys	Thr	His
Thr	Cys 690	Pro	Pro	Сүз	Pro	Ala 695	Pro	Glu	Leu	Leu	Gly 700	Gly	Pro	Ser	Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr 65 70 75 80

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Ser	Val 130	Tyr	Pro	Leu	Ala	Pro 135	Gly	Ser	Ala	Ala	Gln 140	Thr	Asn	Ser	Met
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Val	Thr	Trp	Asn	Ser 165	Gly	Ser	Leu	Ser	Ser 170	Gly	Val	His	Thr	Phe 175	Pro
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Gln 385	Pro	Ala	Glu	Asn	Tyr 390	ГЛа	Asn	Thr	Gln	Pro 395	Ile	Met	Asp	Thr	Asp 400
Gly	Ser	Tyr	Phe	Val 405	Tyr	Ser	Lys	Leu	Asn 410	Val	Gln	Lys	Ser	Asn 415	Trp
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Glu	Thr	Val	Thr 20	Ile	Thr	Cys	Arg	Ala 25	Ser	Glu	Asn	Ile	Tyr 30	Ser	Asn	
Leu	Ala	Trp 35	Tyr	Gln	Gln	Lys	Gln 40	Gly	Lys	Ser	Pro	Gln 45	Leu	Leu	Val	
Tyr	Ala 50	Ala	Thr	Asn	Leu	Ala 55	Asp	Gly	Val	Pro	Ser 60	Arg	Phe	Ser	Gly	
Ser 65	Gly	Ser	Gly	Thr	Gln 70	Phe	Ser	Leu	Lys	Ile 75	Asn	Ser	Leu	Gln	Ser 80	
Glu	Asp	Phe	Gly	Thr 85	Tyr	Tyr	Сув	Gln	His 90	Phe	Trp	Gly	Thr	Pro 95	Tyr	
Thr	Phe	Gly	Gly 100	Gly	Thr	Lys	Leu	Glu 105	Ile	Lys	Arg	Ala	Asp 110	Ala	Ala	
Pro	Thr	Val 115	Ser	Ile	Phe	Pro	Pro 120	Ser	Ser	Glu	Gln	Leu 125	Thr	Ser	Gly	
Gly	Ala 130	Ser	Val	Val	Суз	Phe 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	ГЛа	Asp	Ile	
Asn 145	Val	Lys	Trp	ГЛЗ	Ile 150	Asp	Gly	Ser	Glu	Arg 155	Gln	Asn	Gly	Val	Leu 160	
Asn	Ser	Trp	Thr	Asp 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Met 175	Ser	
Ser	Thr	Leu	Met 180	Leu	Thr	Lys	Asp	Glu 185	Tyr	Glu	Arg	His	Asn 190	Ser	Tyr	
Thr	Cys	Glu 195	Ala	Thr	His	Lys	Thr 200	Ser	Thr	Ser	Pro	Ile 205	Val	Гла	Ser	
Phe	Asn 210	Arg	Asn	Glu	Cys											

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<210> SEQ ID NO 126 <211> LENGTH: 1386

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<212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (2B8 Variable Region and IgG1 Constant Region) <400> SEQUENCE: 126 atgggatgga gctatatcat cctctttttg gtagcaacag ctacagatgt ccactcccag 60 gtccaactgc agcageetgg ggetgaactg gtgaageetg ggaetteagt gaagetgtee 120 tgcaaggctt ctggctacac cttcaccacc tactggatgc actgggtgaa tcagaggcct 180 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat 240 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg 300 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt 360 ggtagcatet ttgactaetg gggccaagge accaetetea cagteteete agecaaaaeg 420 acacccccat ctgtctatcc actggcccct ggatctgctg cccaaactaa ctccatggtg 480 accetgggat geetggteaa gggetattte eetgageeag tgaeagtgae etggaaetet 540 ggatccctgt ccagcggtgt gcacaccttc ccagctgtcc tgcagtctga cctctacact 600 ctgagcaget cagtgaetgt cecetecage acetggeeca gegagaeegt caeetgeaae 660 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt 720 tgtaagcott goatatgtac agtocoagaa gtatoatotg tottoatott cooccoaaag 780 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc 840 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca 900 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt 960 1020 cccatcatqc accaqqactq qctcaatqqc aaqqaqttca aatqcaqqqt caacaqtqca gettteeetq ceeccatega gaaaaccate teeaaaacca aaggeagaee gaaggeteea 1080 caggtgtaca ccattecace teccaaggag cagatggeea aggataaagt cagtetgace 1140 1200 tqcatqataa caqacttctt ccctqaaqac attactqtqq aqtqqcaqtq gaatqqqcaq ccagcggaga actacaagaa cactcagccc atcatggaca cagatggctc ttacttcgtc 1260 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct 1320 gtgttacatg agggcctgca caaccaccat actgagaaga gcctctccca ctctcctggt 1380 aaatqa 1386 <210> SEQ ID NO 127 <211> LENGTH: 442 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (2B8 Variable Region and IgG1 Constant Region) <400> SEQUENCE: 127 Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Thr 10 Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr Trp Met His Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile 35 40 45 Gly Glu Ile Asn Pro Thr Asn Gly His Thr Asn Tyr Asn Glu Lys Phe

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	50					55					60				
Lys 65	Ser	Lys	Ala	Thr	Leu 70	Thr	Val	Asp	Lys	Ser 75	Ser	Ser	Thr	Ala	Tyr 80
Met	Gln	Leu	Ser	Ser 85	Leu	Thr	Ser	Glu	Asp 90	Ser	Ala	Val	Tyr	Tyr 95	Суз
Ala	Arg	Asn	Tyr 100	Val	Gly	Ser	Ile	Phe 105	Asp	Tyr	Trp	Gly	Gln 110	Gly	Thr
Thr	Leu	Thr 115	Val	Ser	Ser	Ala	Lys 120	Thr	Thr	Pro	Pro	Ser 125	Val	Tyr	Pro
Leu	Ala 130	Pro	Gly	Ser	Ala	Ala 135	Gln	Thr	Asn	Ser	Met 140	Val	Thr	Leu	Gly
Cys 145	Leu	Val	Lys	Gly	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Thr	Trp	Asn 160
Ser	Gly	Ser	Leu	Ser 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
Ser	Asp	Leu	Tyr 180	Thr	Leu	Ser	Ser	Ser 185	Val	Thr	Val	Pro	Ser 190	Ser	Thr
Trp	Pro	Ser 195	Glu	Thr	Val	Thr	Cys 200	Asn	Val	Ala	His	Pro 205	Ala	Ser	Ser
Thr	Lys 210	Val	Asp	LYa	Lys	Ile 215	Val	Pro	Arg	Asp	Cys 220	Gly	Суз	ГЛа	Pro
Cys 225	Ile	Суз	Thr	Val	Pro 230	Glu	Val	Ser	Ser	Val 235	Phe	Ile	Phe	Pro	Pro 240
Lys	Pro	Lys	Asp	Val 245	Leu	Thr	Ile	Thr	Leu 250	Thr	Pro	Lys	Val	Thr 255	Сүз
Val	Val	Val	Asp 260	Ile	Ser	ГÀа	Asp	Asp 265	Pro	Glu	Val	Gln	Phe 270	Ser	Trp
Phe	Val	Asp 275	Asp	Val	Glu	Val	His 280	Thr	Ala	Gln	Thr	Gln 285	Pro	Arg	Glu
Glu	Gln 290	Phe	Asn	Ser	Thr	Phe 295	Arg	Ser	Val	Ser	Glu 300	Leu	Pro	Ile	Met
His 305	Gln	Asp	Trp	Leu	Asn 310	Gly	Lys	Glu	Phe	Lys 315	Сүз	Arg	Val	Asn	Ser 320
Ala	Ala	Phe	Pro	Ala 325	Pro	Ile	Glu	ГЛа	Thr 330	Ile	Ser	Lys	Thr	Lys 335	Gly
Arg	Pro	Lys	Ala 340	Pro	Gln	Val	Tyr	Thr 345	Ile	Pro	Pro	Pro	Lys 350	Glu	Gln
Met	Ala	Lys 355	Asp	Lys	Val	Ser	Leu 360	Thr	Сув	Met	Ile	Thr 365	Asp	Phe	Phe
Pro	Glu 370	Asp	Ile	Thr	Val	Glu 375	Trp	Gln	Trp	Asn	Gly 380	Gln	Pro	Ala	Glu
Asn 385	Tyr	Lys	Asn	Thr	Gln 390	Pro	Ile	Met	Asp	Thr 395	Asp	Gly	Ser	Tyr	Phe 400
Val	Tyr	Ser	Lys	Leu 405	Asn	Val	Gln	Lys	Ser 410	Asn	Trp	Glu	Ala	Gly 415	Asn
Thr	Phe	Thr	Cys 420	Ser	Val	Leu	His	Glu 425	Gly	Leu	His	Asn	His 430	His	Thr
Glu	Lys	Ser 435	Leu	Ser	His	Ser	Pro 440	Gly	Lya						

<210> SEQ ID NO 128 <211> LENGTH: 705 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence

<220 <223)> FI > 0] Re	EATUF THER egion	RE: INFC n and	RMAI d Cor	ION: nstar	Ful nt Re	l Le egior	ength 1)	ı Lig	jht (Chair	n (2E	38 Ka	appa	Varia	able	
<400	> SE	QUEN	ICE :	128													
atgg	jaato	cac a	agact	ctg	gt ct	tcat	atco	c ata	actgo	ctct	ggtt	atat	ag t	gcto	gatgg	g	60
aaca	ittgi	caa t	gaco	ccaat	c to	cccaa	aatco	c ato	gtcca	atgt	cagt	agga	aga 🤉	gaggo	gtcac	с	120
ttga	ıgcto	gca a	aggco	cagto	ga ga	aatgt	ggtt	t t ct	tato	gtat	cctç	ggtat	cca a	acaga	aacc	a	180
gcgc	agto	ete d	ctaaa	actgo	et ga	atata	acggg	g gca	atcca	aacc	ggaa	acact	ag a	ggtco	cccga	t	240
cgct	tcad	cag g	gcagt	ggat	c tç	gcaad	cagat	t tt	cacto	ctga	ccat	cago	cag t	gtgo	adda.	t	300
gaag	jacct	tg d	cagat	tato	ca ct	gtgg	ggcag	g agt	taca	aact	atco	cgta	cac q	gttco	gagg	g	360
ggga	ccaç	ggc t	ggaa	aataa	aa ad	cgggg	ctgat	: gct	gcad	ccaa	ctgt	atco	cat 🤇	cttco	cacc	a	420
tcca	ıgtga	agc a	agtta	aacat	c tç	ggago	gtgco	c tca	agtco	gtgt	gctt	ctt	gaa (caact	tcta	с	480
ccca	aaga	aca t	caat	gtca	aa gt	ggaa	agatt	: gat	ggca	agtg	aaco	gacaa	aaa 1	ggcó	gteet	g	540
aaca	gtt	gga (ctgat	cago	ga ca	agcaa	aagad	c ago	cacct	caca	gcat	gago	cag (cacco	ctcac	g	600
ttga	iccaa	agg a	acgag	gtato	ga ac	cgaca	ataad	ago	tata	acct	gtga	aggeo	cac 1	ccaca	agac	a	660
tcaa	ctto	cac d	ccatt	gtca	aa ga	agctt	caad	ago	gaato	gagt	gtta	ag					705
<210 <211 <212 <213 <220 <223	 > SE > LE > TY > OF > FE > OI Re > SE 	EQ II ENGTH PE: RGANI EATUF THER egion) NO H: 21 PRT SM: E: INFC n and NCE:	129 .4 Arti DRMAI d Cor 129	fici ION: hstar	al S Ful nt Re	Seque 1 Le egior	ence ength	ı Lig	Jht (Chair	n (2E	38 Ka	appa	Varia	able	
Asn 1	Ile	Val	Met	Thr 5	Gln	Ser	Pro	Lys	Ser 10	Met	Ser	Met	Ser	Val 15	Gly		
Glu	Arg	Val	Thr 20	Leu	Ser	Сув	Lys	Ala 25	Ser	Glu	Asn	Val	Val 30	Ser	Tyr		
Val	Ser	Trp 35	Tyr	Gln	Gln	Lys	Pro 40	Ala	Gln	Ser	Pro	Lys 45	Leu	Leu	Ile		
Tyr	Gly 50	Ala	Ser	Asn	Arg	Asn 55	Thr	Gly	Val	Pro	Asp 60	Arg	Phe	Thr	Gly		
Ser 65	Gly	Ser	Ala	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Val	Arg	Ala 80		
Glu	Asp	Leu	Ala	Asp 85	Tyr	His	Cys	Gly	Gln 90	Ser	Tyr	Asn	Tyr	Pro 95	Tyr		
Thr	Phe	Gly	Gly 100	Gly	Thr	Arg	Leu	Glu 105	Ile	Lys	Arg	Ala	Asp 110	Ala	Ala		
Pro	Thr	Val 115	Ser	Ile	Phe	Pro	Pro 120	Ser	Ser	Glu	Gln	Leu 125	Thr	Ser	Gly		
Gly	Ala 130	Ser	Val	Val	Суз	Phe 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Lys	Asp	Ile		
Asn 145	Val	Lys	Trp	Lys	Ile 150	Asp	Gly	Ser	Glu	Arg 155	Gln	Asn	Gly	Val	Leu 160		
Asn	Ser	Trp	Thr	Asp 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Met 175	Ser		
Ser	Thr	Leu	Thr 180	Leu	Thr	Lys	Asp	Glu 185	Tyr	Glu	Arg	His	Asn 190	Ser	Tyr		
Thr	Cys	Glu 195	Ala	Thr	His	Lys	Thr 200	Ser	Thr	Ser	Pro	Ile 205	Val	Lys	Ser		

Phe Asn Arg Asn Glu Cys 210

<210> SEO ID NO 130 <211> LENGTH: 1386 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (2F8 Variable Region and IgG1 Constant Region) <400> SEQUENCE: 130 atggaatgga getgggtett tetetteete etgteagtaa etgeaggtgt eeaetgeeag 60 gtccagctga agcagtctgg agctgagctg gtgaggcctg ggacttcagt gaagatgtcc 120 tgcaaggett etggetacae etteactaee taetatatae aetgggtgaa teagaggeet 180 ggacagggcc ttgagtggat tggaaagatt ggtcctggaa gtggtagtac ttactacaat 240 gagatgttca aagacaaggc cacattgact gtagacacat cctccagcac agcctacatg 300 cageteagea geetgacate tgaegaetet geggtetatt tetgtgeaag aaggggaetg 360 ggacgtggct ttgactactg gggccaaggc accactctca cagtctcctc agccaaaacg 420 acacccccat ctgtctatcc actggcccct ggatctgctg cccaaactaa ctccatggtg 480 accotgggat gootggtcaa gggctattto cotgagocag tgacagtgac otggaactot 540 ggatecetgt ccageggtgt geacacette ccagetgtee tgeagtetga eetetaeaet 600 ctgagcaget cagtgaetgt cecetecage acetggeeca gegagaeegt cacetgeaae 660 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt 720 tqtaaqcctt qcatatqtac aqtcccaqaa qtatcatctq tcttcatctt ccccccaaaq 780 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc 840 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca 900 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt 960 cccatcatgc accaggactg gctcaatggc aaggagttca aatgcagggt caacagtgca 1020 gettteeetg eccecatega gaaaaceate teeaaaacea aaggeagaee gaaggeteea 1080 caggtgtaca ccattccacc tcccaaggag cagatggcca aggataaagt cagtctgacc 1140 tgcatgataa cagacttctt ccctgaagac attactgtgg agtggcagtg gaatgggcag 1200 ccagcggaga actacaagaa cactcagccc atcatggaca cagatggctc ttacttcgtc 1260 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct 1320 gtgttacatg agggcctgca caaccaccat actgagaaga gcctctccca ctctcctggt 1380 aaatga 1386 <210> SEQ ID NO 131 <211> LENGTH: 442 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (2F8 Variable Region and IgG1 Constant Region) <400> SEQUENCE: 131 Gln Val Gln Leu Lys Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr 1 10 Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr 20 25 30

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Tyr	Ile	His 35	Trp	Val	Asn	Gln	Arg 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Ile
Gly	Lys 50	Ile	Gly	Pro	Gly	Ser 55	Gly	Ser	Thr	Tyr	Tyr 60	Asn	Glu	Met	Phe
Lys 65	Aab	Lys	Ala	Thr	Leu 70	Thr	Val	Aab	Thr	Ser 75	Ser	Ser	Thr	Ala	Tyr 80
Met	Gln	Leu	Ser	Ser 85	Leu	Thr	Ser	Aab	Asp 90	Ser	Ala	Val	Tyr	Phe 95	Сув
Ala	Arg	Arg	Gly 100	Leu	Gly	Arg	Gly	Phe 105	Asp	Tyr	Trp	Gly	Gln 110	Gly	Thr
Thr	Leu	Thr 115	Val	Ser	Ser	Ala	Lys 120	Thr	Thr	Pro	Pro	Ser 125	Val	Tyr	Pro
Leu	Ala 130	Pro	Gly	Ser	Ala	Ala 135	Gln	Thr	Asn	Ser	Met 140	Val	Thr	Leu	Gly
Cys 145	Leu	Val	Lys	Gly	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Thr	Trp	Asn 160
Ser	Gly	Ser	Leu	Ser 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
Ser	Asp	Leu	Tyr 180	Thr	Leu	Ser	Ser	Ser 185	Val	Thr	Val	Pro	Ser 190	Ser	Thr
Trp	Pro	Ser 195	Glu	Thr	Val	Thr	Cys 200	Asn	Val	Ala	His	Pro 205	Ala	Ser	Ser
Thr	Lys 210	Val	Asp	Lys	Lys	Ile 215	Val	Pro	Arg	Asp	Cys 220	Gly	Суз	Lys	Pro
Cys 225	Ile	Cys	Thr	Val	Pro 230	Glu	Val	Ser	Ser	Val 235	Phe	Ile	Phe	Pro	Pro 240
Lys	Pro	Lys	Asp	Val 245	Leu	Thr	Ile	Thr	Leu 250	Thr	Pro	Lys	Val	Thr 255	Суз
Val	Val	Val	Asp 260	Ile	Ser	Lys	Asp	Asp 265	Pro	Glu	Val	Gln	Phe 270	Ser	Trp
Phe	Val	Asp 275	Asp	Val	Glu	Val	His 280	Thr	Ala	Gln	Thr	Gln 285	Pro	Arg	Glu
Glu	Gln 290	Phe	Asn	Ser	Thr	Phe 295	Arg	Ser	Val	Ser	Glu 300	Leu	Pro	Ile	Met
His 305	Gln	Asp	Trp	Leu	Asn 310	Gly	Lys	Glu	Phe	Lys 315	Сүз	Arg	Val	Asn	Ser 320
Ala	Ala	Phe	Pro	Ala 325	Pro	Ile	Glu	Lys	Thr 330	Ile	Ser	Lys	Thr	Lys 335	Gly
Arg	Pro	Lys	Ala 340	Pro	Gln	Val	Tyr	Thr 345	Ile	Pro	Pro	Pro	Lys 350	Glu	Gln
Met	Ala	Lys 355	Asp	Lys	Val	Ser	Leu 360	Thr	Суз	Met	Ile	Thr 365	Asp	Phe	Phe
Pro	Glu 370	Asp	Ile	Thr	Val	Glu 375	Trp	Gln	Trp	Asn	Gly 380	Gln	Pro	Ala	Glu
Asn 385	Tyr	Lys	Asn	Thr	Gln 390	Pro	Ile	Met	Aab	Thr 395	Asp	Gly	Ser	Tyr	Phe 400
Val	Tyr	Ser	Гла	Leu 405	Asn	Val	Gln	Lys	Ser 410	Asn	Trp	Glu	Ala	Gly 415	Asn
Thr	Phe	Thr	Cys 420	Ser	Val	Leu	His	Glu 425	Gly	Leu	His	Asn	His 430	His	Thr
Glu	Lys	Ser 435	Leu	Ser	His	Ser	Pro 440	Gly	Lys						

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<210> SEQ ID NO 132 <211> LENGTH: 717 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (2F8 Kappa Variable Region and Constant Region) <400> SEOUENCE: 132 atggagacag acacaateet getatgggtg etgetgetet gggtteeagg etceaetggt gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc 120 atctcctgca aggccagcca aagtgttgat tatgatggta atagttatat caactggtac 180 caacagaaac caggacagcc acccaaagtc ctcatctatg ttgcatccaa tctagaatct 240 gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat 300 cctgtggagg aggaggatgc tgcaacctat tactgtcagc aaagtattga ggatcctccc 360 acgttcggtg ctgggaccaa gctggagctg aaacgggctg atgctgcacc aactgtatcc 420 atetteceae catecagtga geagttaaca tetggaggtg eetcagtegt gtgettettg 480 aacaacttct accccaaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa 540 aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc 600 agcaccetea egttgaceaa ggaegagtat gaaegaeata aeagetatae etgtgaggee 660 actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgttag 717 <210> SEQ ID NO 133 <211> LENGTH: 218 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (2F8 Kappa Variable Region and Constant Region) <400> SEOUENCE: 133 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly 1 5 10 15 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp 20 25 30 Gly Asn Ser Tyr Ile Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro 40 35 45 Lys Val Leu Ile Tyr Val Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala 50 55 60 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His 70 75 65 80 Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Ile 85 90 95 Glu Asp Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg 105 100 110 Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln 115 120 125 Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr 130 135 140 Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln 155 145 150 160 Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr 165 170 175

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Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg 180 185 190
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro 195 200 205
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys 210 215
<210> SEQ ID NO 134 <211> LENGTH: 1392 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (3B6 Variable Region and IgG1 Constant Region)
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tgcaaggett etggetatgt atteagtage taetggatga aetgggtgaa geagaggeet 180
ggacagggtc ttgagtggat tggacagatt tatcctggag atggtgatag taactacaat 240
ggaaacttca agggtaaagc cacactgact gcagacaaat cctccagtac agcctacatg 300
cageteagea geetaacate tgaggaetet geggtetatt tetgtgeate ceageteggg 360
ctacgtgaga actactttga ctactggggc caaggcacca ctctcacagt ctcctcagcc 420
aaaacgacac ccccatctgt ctatccactg gcccctggat ctgctgccca aactaactcc 480
atggtgaccc tgggatgcct ggtcaagggc tatttccctg agccagtgac agtgacctgg 540
aactetggat eeetgteeag eggtgtgeae acetteeeag etgteetgea gtetgaeete 600
tacactetga geageteagt gaetgteece teeageaeet ggeeeagega gaeegteaee 660
tgcaacgttg cccacccggc cagcagcacc aaggtggaca agaaaattgt gcccagggat 720
tgtggttgta agcettgeat atgtacagte eeagaagtat eatetgtett eatetteeee 780
ccaaagccca aggatgtgct caccattact ctgactccta aggtcacgtg tgttgtggta 840
gacatcagca aggatgatcc cgaggtccag ttcagctggt ttgtagatga tgtggaggtg 900
cacacagete agaegeaace eegggaggag cagtteaaca geaettteeg eteagteagt 960
gaactteeca teatgeacea ggaetggete aatggeaagg agtteaaatg eagggteaae 1020
agtgcagett teeetgeeee categagaaa accateteea aaaccaaagg cagaeegaag 1080
getecacagg tgtacaccat tecacetece aaggageaga tggeeaagga taaagteagt 1140
ctgacctgca tgataacaga cttcttccct gaagacatta ctgtggagtg gcagtggaat 1200
gggcagccag cggagaacta caagaacact cagcccatca tggacacaga tggctcttac 1260
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cctggtaaat ga 1392
<210> SEQ ID NO 135 <211> LENGTH: 444 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (3B6 Variable Region and IgG1 Constant egion)

<400> SEQUENCE: 135

Gln 1	Val	Gln	Leu	Gln 5	Gln	Ser	Gly	Ala	Glu 10	Leu	Val	Arg	Pro	Gly 15	Ser
Ser	Val	Lys	Ile 20	Ser	Cya	Lys	Ala	Ser 25	Gly	Tyr	Val	Phe	Ser 30	Ser	Tyr
Trp	Met	Asn 35	Trp	Val	Lys	Gln	Arg 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Ile
Gly	Gln 50	Ile	Tyr	Pro	Gly	Asp 55	Gly	Aab	Ser	Asn	Tyr 60	Asn	Gly	Asn	Phe
Lys 65	Gly	Lys	Ala	Thr	Leu 70	Thr	Ala	Aab	Lys	Ser 75	Ser	Ser	Thr	Ala	Tyr 80
Met	Gln	Leu	Ser	Ser 85	Leu	Thr	Ser	Glu	Asp 90	Ser	Ala	Val	Tyr	Phe 95	Сув
Ala	Ser	Gln	Leu 100	Gly	Leu	Arg	Glu	Asn 105	Tyr	Phe	Asp	Tyr	Trp 110	Gly	Gln
Gly	Thr	Thr 115	Leu	Thr	Val	Ser	Ser 120	Ala	Lys	Thr	Thr	Pro 125	Pro	Ser	Val
Tyr	Pro 130	Leu	Ala	Pro	Gly	Ser 135	Ala	Ala	Gln	Thr	Asn 140	Ser	Met	Val	Thr
Leu 145	Gly	Суз	Leu	Val	Lys 150	Gly	Tyr	Phe	Pro	Glu 155	Pro	Val	Thr	Val	Thr 160
Trp	Asn	Ser	Gly	Ser 165	Leu	Ser	Ser	Gly	Val 170	His	Thr	Phe	Pro	Ala 175	Val
Leu	Gln	Ser	Asp 180	Leu	Tyr	Thr	Leu	Ser 185	Ser	Ser	Val	Thr	Val 190	Pro	Ser
Ser	Thr	Trp 195	Pro	Ser	Glu	Thr	Val 200	Thr	Cys	Asn	Val	Ala 205	His	Pro	Ala
Ser	Ser 210	Thr	Lys	Val	Asb	Lys 215	Lys	Ile	Val	Pro	Arg 220	Asb	Сүз	Gly	Суз
Lys 225	Pro	Сүз	Ile	CÀa	Thr 230	Val	Pro	Glu	Val	Ser 235	Ser	Val	Phe	Ile	Phe 240
Pro	Pro	Lys	Pro	Lys 245	Asp	Val	Leu	Thr	Ile 250	Thr	Leu	Thr	Pro	Lys 255	Val
Thr	Сув	Val	Val 260	Val	Asp	Ile	Ser	Lys 265	Asp	Asp	Pro	Glu	Val 270	Gln	Phe
Ser	Trp	Phe 275	Val	Asp	Asp	Val	Glu 280	Val	His	Thr	Ala	Gln 285	Thr	Gln	Pro
Arg	Glu 290	Glu	Gln	Phe	Asn	Ser 295	Thr	Phe	Arg	Ser	Val 300	Ser	Glu	Leu	Pro
Ile 305	Met	His	Gln	Asp	Trp 310	Leu	Asn	Gly	Lys	Glu 315	Phe	Lys	Сүз	Arg	Val 320
Asn	Ser	Ala	Ala	Phe 325	Pro	Ala	Pro	Ile	Glu 330	Lys	Thr	Ile	Ser	Lys 335	Thr
Lys	Gly	Arg	Pro 340	Lys	Ala	Pro	Gln	Val 345	Tyr	Thr	Ile	Pro	Pro 350	Pro	Lys
Glu	Gln	Met 355	Ala	Lys	Asp	ГÀа	Val 360	Ser	Leu	Thr	Сүз	Met 365	Ile	Thr	Asp
Phe	Phe 370	Pro	Glu	Asp	Ile	Thr 375	Val	Glu	Trp	Gln	Trp 380	Asn	Gly	Gln	Pro
Ala 385	Glu	Asn	Tyr	Lys	Asn 390	Thr	Gln	Pro	Ile	Met 395	Asp	Thr	Asp	Gly	Ser 400
Tyr	Phe	Val	Tyr	Ser 405	Lys	Leu	Asn	Val	Gln 410	Lys	Ser	Asn	Trp	Glu 415	Ala
Gly	Asn	Thr	Phe	Thr	Cys	Ser	Val	Leu	His	Glu	Gly	Leu	His	Asn	His

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145 150 155 160
Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
Ser Thr Leu Thr Lvs Asp Glu Tvr Glu Ara His Asp Ser Tvr
180 185 190
Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser 195 200 205
Phe Asn Arg Asn Glu Cys 210
<210> SEQ ID NO 138 <211> LENGTH: 1361 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (3D11 Variable Region and IgG1 Constant Region)
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gtacagetga aggagteagg acetggeetg gtggegeeet eacagageet gteeateaet 120
tgcactgtct ctgggttttc attaaccagc tatagtttac actgggttcg ccagcctcca 180
ggaaagggtc tggaatggct gggagtaata tgggctggtg gaaacacaaa ttataattcg 240
teteteatgt eeagaetgae cateaggaaa gacaaeteea agageeaagt tttettaaaa 300
atgaacagte tgeaaactga tgacacagee atgtactaet gtgeeagaga gaggtttget 360
tactggggcc aagggactet ggteaetgte tetgeageea aaaegaeaee eeeatetgte 420
tatccactgg cccctggatc tgctgcccaa actaactcca tggtgaccct gggatgcctg 480
gtcaaggget attteeetga gecagtgaca gtgaeetgga aetetggate eetgteeage 540
ggtgtgcaca cetteceage tgteetgeag tetgaeetet acaetetgag cageteagtg 600
actgtcccct ccagcacctg gcccagcgag accgtcacct gcaacgttgc ccacccggcc 660
agcagcacca aggtggacaa gaaaattgtg cccagggatt gtggttgtaa gccttgcata 720
tgtacagtcc cagaagtatc atctgtcttc atcttccccc caaagcccaa ggatgtgctc 780
accattactc tgactcctaa ggtcacgtgt gttgtggtag acatcagcaa ggatgatccc 840
gaggtccagt tcagctggtt tgtagatgat gtggaggtgc acacagctca gacgcaaccc 900
cgggaggagc agttcaacag cactttccgc tcagtcagtg aacttcccat catgcaccag 960
gactggctca atggcaagga gttcaaatgc agggtcaaca gtgcagcttt ccctgccccc 1020
atcgagaaaa ccatctccaa aaccaaaggc agaccgaagg ctccacaggt gtacaccatt 1080
ccacctccca aggagcagat ggccaaggat aaagtcagtc tgacctgcat gataacagac 1140
ttcttccctg aagacattac tgtggagtgg cagtggaatg ggcagccagc ggagaactac 1200
aagaacactc agcccatcat ggacacagat ggctcttact tcgtctacag caagctcaat 1260
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<210> SEQ ID NO 139 <211> LENGTH: 437 <212> TYPE: PRT <212> OPCANLEW. Attificial Sequence

<213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Heavy Chain (3D11 Variable Region and IgG1 Constant Region)
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<400> SEOUENCE: 139 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr 2.0 Ser Leu His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Ser Leu Met Ser Arg Leu Thr Ile Arg Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg Glu Arg Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly 130 135 140 Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys 195 200 205 Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser

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405 410	415
Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Se 420 425 43	er Leu Ser 30
His Ser Pro Gly Lys 435	
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agaggacaaa ttgttctcac ccagtctcca gcaatcatgt ctgcatatco	c aggggagaag 120
gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgcactggta	a ccagcagaag 180
tcaggcacct cccccaaaag atggatttat gacacatcca aactggctto	c tggagtccct 240
getegettea gtggeagtgg gtetgggaee tettaeteee teacaateag	g tagtatggag 300
gctgaagatg ctgccactta ttactgccag cagtggagta gtaacccact	cacgttcggt 360
gctgggacca agctggagct gaaacgggct gatgctgcac caactgtatc	c catcttccca 420
ccatccagtg agcagttaac atctggaggt gcctcagtcg tgtgcttctt	gaacaacttc 480
taccccaaag acatcaatgt caagtggaag attgatggca gtgaacgaca	a aaatggcgtc 540
ctgaacagtt ggactgatca ggacagcaaa gacagcacct acagcatgag	g cagcaccete 600
acgttgacca aggacgagta tgaacgacat aacagctata cctgtgaggc	c cactcacaag 660
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<210> SEQ ID NO 141 <211> LENGTH: 213 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (3D11 Region and Constant Region) <400> SEQUENCE: 141	. Kappa Variable
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1 5 10	15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Se 20 25 30	er Tyr Met)
His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Tr 35 40 45	rp Ile Tyr
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Se 50 55 60	er Gly Ser
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Gl 65 70 75	lu Ala Glu 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pr 85 90	ro Leu Thr 95
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Al 100 105 11	La Ala Pro LO
Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Se 115 120 125	er Gly Gly
Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys As	sp Ile Asn

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											-	cont	tin	ued				
	130					135					140							
Val 145	Lys	Trp	Lys	Ile	Asp 150	Gly	Ser	Glu	Arg	Gln 155	Asn	Gly	Val	Leu	Asn 160			
Ser	Trp	Thr	Asp	Gln 165	Asp	Ser	Lys	Asp	Ser 170	Thr	Tyr	Ser	Met	Ser 175	Ser			
Thr	Leu	Thr	Leu 180	Thr	Lys	Asp	Glu	Tyr 185	Glu	Arg	His	Asn	Ser 190	Tyr	Thr			
Суз	Glu	Ala 195	Thr	His	Lys	Thr	Ser 200	Thr	Ser	Pro	Ile	Val 205	Lys	Ser	Phe			
Asn	Arg 210	Asn	Glu	СЛа														
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atga	acti	~ ta	aacto	ragat	t. az	attt	cett	ata	cette	attt	taaa	aaat	at a	aadt	ataa	a	60	
ata	radet	. aa 1	taaa	atete	na a	naaa	actta	ata	acad	rcta	aaa	atco	ret d	1222	tete	ic i	120	
tata	acado	ct (ctaa	attca	ac ti	ttcad	ataac	tat	ttaca	atat	ctto	aatt		ccada	actco	a	180	
aaaa	agac	aac 1	t.agad	at.aa	at co	acata	acatt	adt	tagto	aata	atad	at.ago	ac d	tact	atec	a 2	240	
gaca	atat	a a	aaaat	cqat	t ca	accat	ctcc	ca:	agaca	aatq	ccaa	adaad	cac o	cctat	acct	a 3	300	
caaa	ataac	ica d	atete	raaqt	te te	aada;	acaca		atai	tatt	acto	atata	ad a	acaac	aaaa	t 3	360	
aatt	atta	aca d	aaaa	ctato	ac ta	ataa	actac	ta	aat	caaq	gaad	ctca	aat d	catco	atete	c 4	120	
tcad	accaa	iaa (cqaca	accco	cc at	tctq	ctat	. cca	actq	accc	ctq	atct	ac 1	acco	aaac	t 4	180	
aact	ccat	aq 1	tqaco	ectqo	aq at	tqcct	caato	aad	aaaci	tatt	tccd	tqac	acc a	aqtqa	acaqt	a :	540	
acct	qqaa	act of	ctqqa	atcco	t qt	tccad	acqat	qto	qcaca	acct	tcco	caqct	qt (cctq	aqto	t e	500	
qaco	tcta	aca (ctcto	aaca	ad ct	tcaqt	act	ato	20001	tcca	qcad	cctad	acc d	caqco	aqac	c é	560	
atca	accto	ica a	acatt	acco	ca co	ccaa	ccaqo	aq	cacca	aaqq	taaa	acaac	jaa a	atto	ataco	c -	720	
aqqq	atto	, ita (gttgt	aaqo	c ti	tqcat	tatqt	aca	aqtco	ccaq	aaqt	atca	atc 1	rgtet	tcat	.c T	780	
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qaqq	atqca	aca (caqct	caqa	ac qu	caaco	ccqq	qaq	qqaq	caqt	tcaa	acaqo	cac 1	ttco	gete	a g	960	
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- qtca	acac	qtq (caqct	ttco	c to	qeee	ccato	qad	qaaaa	acca	tcto	caaa	aac o	caaad	qcac	- 1a 10	080	
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tett	actt	- .cg 1	- tctad	cagea	aa go	ctcaa	atgto	cad	gaaga	- agca	acto	- Jggac	- Jgc a	aggaa	atac	t 13	320	
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la Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val. 50 55 60	
ys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr 5 70 75 80	
eu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Ile Tyr Tyr Cys 85 90 95	
'al Arg Gln Gly Asp Gly Tyr Tyr Gly Asp Tyr Ala Met Asp Tyr Trp 100 105 110	
ly Gln Gly Thr Ser Val Ile Val Ser Ser Ala Lys Thr Thr Pro Pro 115 120 125	
er Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met 130 135 140	
'al Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr .45 150 155 160	
'al Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro 165 170 175	
la Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Val Thr Val. 180 185 190	
ro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His 195 200 205	
ro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys 210 215 220	
ly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe 25 230 235 240	
le Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro 245 250 255	
ys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val 260 265 270	
In Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr 275 280 285	
In Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu 290 295 300	
eu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys 05 310 315 320	
rg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser 325 330 335	
ys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro 340 345 350	
ro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile 355 360 365	
hr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly 370 375 380	

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Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp 390 395 385 400 Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp 410 405 415 Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His 420 425 430 Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys 435 440 445 <210> SEQ ID NO 144 <211> LENGTH: 705 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (1D3 Kappa Variable Region and Constant Region) <400> SEQUENCE: 144 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgtcagatgt gacatecaga tgactcagte tecageetee etatetgtat etgtgggaga aactgteace atcacatgtc gaacaagtga gaatatttac agtaatttag cgtggtatca gcagaaacag ggaaaatctc ctcagctcct aatctatgct gcaacaaact tagcagatgg tgtgccatca aggttcagtg gcagtggatc aggcacacag ttttccctca ggatcaacag cctgcagtct gaagattttg ggaggtatta ctgtcaacat ttttgggggga ctccgtacac gttcggaggg gggaccaaac tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg aacaqttqqa ctqatcaqqa caqcaaaqac aqcacctaca qcatqaqcaq caccctcacq ttqaccaaqq acqaqtatqa acqacataac aqctatacct qtqaqqccac tcacaaqaca tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag <210> SEO ID NO 145 <211> LENGTH: 214 <212> TYPE · PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (1D3 Kappa Variable Region and Constant Region) <400> SEQUENCE: 145 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly 5 10 15 1 Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Glu Asn Ile Tyr Ser Asn 20 25 30 Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile 35 40 45 Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Gln Phe Ser Leu Arg Ile Asn Ser Leu Gln Ser 70 75 65 80 Glu Asp Phe Gly Arg Tyr Tyr Cys Gln His Phe Trp Gly Thr Pro Tyr 85 90 95 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala 100 105 110

206

60

120

180

240

300

360

420

480

540

600

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Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	Gln	Leu	Thr	Ser	Gly	
G]	77-	115	**- 7		G	Dl	120	3	3	Dl	T	125 Deve	T	7	T] -	
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Asn	Ser	Trp	Thr	Asp 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Met 175	Ser	
Ser	Thr	Leu	Thr 180	Leu	Thr	Lys	Asp	Glu 185	Tyr	Glu	Arg	His	Asn 190	Ser	Tyr	
Thr	Cya	Glu 195	Ala	Thr	His	Lys	Thr 200	Ser	Thr	Ser	Pro	Ile 205	Val	Lys	Ser	
Phe	Asn 210	Arg	Asn	Glu	Суз											
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Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile 355 360 365 Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly 370 375 380 Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp 385 390 395 400 Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp 405 410 415 Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His 425 420 430 Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys 435 440 445 <210> SEQ ID NO 148 <211> LENGTH: 705 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (1F3 Kappa Variable Region and Constant Region) <400> SEQUENCE: 148 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt 60 gacatecaga tgactcagte tecageetee etatetgtat etgtgggaga aactgteace 120 atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag 180 ggaaaatctc ctcagctcct ggtctatgat gcaacacact taccagatgg tgtgccatca 240 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct 300 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gtttggaggg 360 gggaccagac tggaaattaa acgggctgat gctgcaccaa ctgtatccat cttcccacca 420 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac 480 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg 540 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg 600 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca 660 705 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag <210> SEQ ID NO 149 <211> LENGTH: 214 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Full Length Light Chain (1F3 Kappa Variable Region and Constant Region) <400> SEOUENCE: 149 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly 5 10 15 Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Asn 20 25 30 Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val 35 40 45 Tyr Asp Ala Thr His Leu Pro Asp Gly Val Pro Ser Arg Phe Ser Gly 55 60 Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Ser 70 75 65 80

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Arg	Val	Asn	Ser	Ala	Ala	Phe	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser		

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Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile 355 360 365	
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Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp 385 390 395 400	
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His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240	
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Thr	Pro	Glu	Val 260	Thr	Суз	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro	
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Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	

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on	E.	Т	n	11	e	a	

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Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr 325 330 335	
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu 340 345 350	
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Tyr	Gly 50	Ala	Ser	Asn	Arg	Asn 55	Thr	Gly	Val	Pro	60 60	Arg	Phe	Thr	Gly	
Ser 65	Gly	Ser	Ala	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Val	Arg	Ala 80	
Glu	Asp	Leu	Ala	Asp 85	Tyr	His	Cys	Gly	Gln 90	Ser	Tyr	Asn	Tyr	Pro 95	Tyr	
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Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly	
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Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser	
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atq	acto	aca d	ectad	aaaa	at co	etect	ctto	a ato	acac	icad	ctad	aaa	ac d	caco	accaaa	60
atco	caqct	aa t	acad	atcto	a ac	acta	aato	aac	aaqo	ctq	aaa	ctaca	aqt d	iaaaa	atctcc	120
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Trp	Met	His 35	Trp	Val	Gln	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Met	
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Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser 5 55 70 75	Chr Asp Thr Ala Tyr 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr 2 85 90	Ala Val Tyr Tyr Cys 95
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Tyr	Ile	Cys	Asn	Val 85	Asn	His	Lys	Pro	Ser 90	Asn	Thr	Lys	Val	Asp 95	Lys
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Gln 225	Pro	Arg	Glu	Pro	Gln 230	Val	Tyr	Thr	Leu	Pro 235	Pro	Ser	Arg	Asp	Glu 240
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Pro	Ser	Asp	Ile 260	Ala	Val	Glu	Trp	Glu 265	Ser	Asn	Gly	Gln	Pro 270	Glu	Asn
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Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	Суз	Asp	Lys	Thr	
His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240	
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg	
Thr	Pro	Glu	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro	
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala	
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val	
Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320	
Lys	Cys	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr	
Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu	
Pro	Pro	Ser 355	Arg	Asp	Glu	Leu	Thr 360	ГЛа	Asn	Gln	Val	Ser 365	Leu	Thr	Суз	
Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser	
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400	
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser	
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala	
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tata	aaaa	ntt d	taa	atac	an ct	tta	rcaco	, tar	taa	atac	acto	aato	ica (rcada	ataccc	180
aaa		, , , , , , , , , , , , , , , , , , ,	- aas	ataci	at or	adaa	agatt	- 22t	- cgya	acce	acco	at cet	ac i	taact	acaat	240
222,	cott		aada	cace	at c	acca:	tetez	a act	gaca	aaat	ccat	caa	cac 1	tacat	accto	300
cad	adad	aca d	accte	aad	ac ct	caa	acaco	. acc	ator	att	acto	ataco	aa a	aaact	atatt	360
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gaco	ggeto	ect t	cctto	cctci	ca ca	agcaa	ageto	c aco	gtg	gaca	agaq	gcago	gtg 🤅	gcago	cagggg	1320
aaco	gtett	ct d	catgo	ctccç	gt ga	atgea	atgaç	g gct	ctgo	caca	acca	actad	cac q	gcaga	aagagc	1380
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Trp	Met	His 35	Trp	Val	Arg	Gln	Met 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Met	
Gly	Glu 50	Ile	Asn	Pro	Thr	Asn 55	Gly	His	Thr	Asn	Tyr 60	Asn	Pro	Ser	Phe	
Gln 65	Gly	His	Val	Thr	Ile 70	Ser	Ala	Asp	Lys	Ser 75	Ile	Ser	Thr	Ala	Tyr 80	
Leu	Gln	Trp	Ser	Ser 85	Leu	Lys	Ala	Ser	Asp 90	Thr	Ala	Met	Tyr	Tyr 95	Суа	
Ala	Arg	Asn	Tyr 100	Val	Gly	Ser	Ile	Phe 105	Asp	Tyr	Trp	Gly	Gln 110	Gly	Thr	
Leu	Val	Thr 115	Val	Ser	Ser	Ala	Ser 120	Thr	Lys	Gly	Pro	Ser 125	Val	Phe	Pro	
Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly	
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160	
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln	
Ser	Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser 185	Val	Val	Thr	Val	Pro 190	Ser	Ser	
Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser	
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	Сүз	Asp	Lys	Thr	
His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240	
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg	
Thr	Pro	Glu	Val 260	Thr	Суз	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro	
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Aab	Gly	Val	Glu	Val 285	His	Asn	Ala	
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val	

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Lys Cys I	ys Val Ser 325	Asn Lys Ala	Leu Pro Ala 330	Pro Ile Glu Lys Thr 335	
Ile Ser I	ys Ala Lys 340	Gly Gln Pro	Arg Glu Pro 345	Gln Val Tyr Thr Leu 350	
Pro Pro S	Ser Arg Asp 155	Glu Leu Thr 360	Lys Asn Gln	Val Ser Leu Thr Cys 365	
Leu Val L 370	ys Gly Phe	Tyr Pro Ser 375	Asp Ile Ala	Val Glu Trp Glu Ser 380	
Asn Gly G 385	Sln Pro Glu	Asn Asn Tyr 390	Lys Thr Thr 395	Pro Pro Val Leu Asp 400	
Ser Asp G	ly Ser Phe 405	Phe Leu Tyr	Ser Lys Leu 410	. Thr Val Asp Lys Ser 415	
Arg Trp G	ln Gln Gly 420	Asn Val Phe	Ser Cys Ser 425	Val Met His Glu Ala 430	
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gtgcagctg	ıg tgcagtct	gg agcagaggt	g aaaaagcccg	gggagtetet gaagatetee 120	
tgtaagggt	t ctggatac	ag ctttaccac	c tactggatgc	actgggtgcg ccagatgccc 180	
gggaaaggc	c tggagtgg	at gggggagat	t aatcctacca	acggtcatac taactacaat 240	
ccgtccttc	c aaggccag	gt caccatctc	a gctgacaagt	ccatcagcac tgcctacctg 300	
cagtggagc	a gcctgaag	gc ctcggacac	c gccatgtatt	actgtgcgag aaactatgtt 360	
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Trp Met H 3	lis Trp Val 5	Arg Gln Met 40	Pro Gly Lys	Gly Leu Glu Trp Met 45	
Gly Glu I 50	le Asn Pro	Thr Asn Gly 55	His Thr Asn	Tyr Asn Pro Ser Phe 60	
Gln Gly G 65	3ln Val Thr	Ile Ser Ala 70	Asp Lys Ser 75	Ile Ser Thr Ala Tyr 80	
Leu Gln I	rp Ser Ser	Leu Lys Ala	Ser Asp Thr	Ala Met Tyr Tyr Cys	

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tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccaga	tgccc 180
gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taact	acaat 240
ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcct	acctg 300
cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaact	atgtt 360
ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcct	ccacc 420
aagggeeeat eggtetteee eetggeacee teeteeaaga geacetetgg gggea	cagcg 480
gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtgga	actca 540
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teceteagea gegtggtgae egtgeeetee ageagettgg geaceeagae etaea	tctgc 660
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaat	cttgt 720
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tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctgg	actcc 1260
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<400> SEQUENCE: 171

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Gly	Glu 50	Ile	Asn	Pro	Thr	Asn 55	Gly	His	Thr	Asn	Tyr 60	Asn	Pro	Ser	Phe
Gln 65	Gly	Gln	Val	Thr	Ile 70	Ser	Ala	Asp	Lys	Ser 75	Ile	Ser	Thr	Ala	Tyr 80
Leu	Gln	Trp	Ser	Ser 85	Leu	Lys	Ala	Ser	90 90	Thr	Ala	Met	Tyr	Tyr 95	Суа
Ala	Arg	Asn	Tyr 100	Val	Gly	Ser	Ile	Phe 105	Asp	Tyr	Trp	Gly	Gln 110	Gly	Thr
Leu	Val	Thr 115	Val	Ser	Ser	Ala	Ser 120	Thr	Lys	Gly	Pro	Ser 125	Val	Phe	Pro
Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
Ser	Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser 185	Val	Val	Thr	Val	Pro 190	Ser	Ser
Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Суз	Asn	Val	Asn	His 205	Lys	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	ГÀа	Ser 220	Сүз	Asp	Lys	Thr
His 225	Thr	Сүз	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg
Thr	Pro	Glu	Val 260	Thr	Сүз	Val	Val	Val 265	Aab	Val	Ser	His	Glu 270	Aab	Pro
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Aab	Gly	Val	Glu	Val 285	His	Asn	Ala
ГЛа	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
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Гла	Суз	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu
Pro	Pro	Ser 355	Arg	Asp	Glu	Leu	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Thr	Суз
Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Aab	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
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Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln 35 40 45	
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser 50 55 60	
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu 65 70 75 80	
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser 85 90 95	
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ggccagggga ccaagctgga gatcaaacga actgtggctg caccatctgt cttcatcttc	420
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teecaggaga gtgteacaga geaggaeage aaggaeagea eetaeageet eageageaee	600
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Tyr	Gly 50	Ala	Ser	Asn	Arg	Asn 55	Thr	Gly	Ile	Pro	Ala 60	Arg	Phe	Ser	Gly	
Ser 65	Gly	Ser	Gly	Thr	Glu 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Leu	Gln	Ser 80	
Glu	Asp	Phe	Ala	Val 85	Tyr	Tyr	Сув	Gly	Gln 90	Ser	Tyr	Asn	Tyr	Pro 95	Tyr	
Thr	Phe	Gly	Gln 100	Gly	Thr	Lys	Leu	Glu 105	Ile	Lys	Arg	Thr	Val 110	Ala	Ala	
Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly	
Thr	Ala 130	Ser	Val	Val	Сүз	Leu 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Arg	Glu	Ala	
Lys 145	Val	Gln	Trp	Lys	Val 150	Asp	Asn	Ala	Leu	Gln 155	Ser	Gly	Asn	Ser	Gln 160	
Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser	
Ser	Thr	Leu	Thr 180	Leu	Ser	Lys	Ala	Asp 185	Tyr	Glu	Lys	His	Lys 190	Val	Tyr	
Ala	Cys	Glu 195	Val	Thr	His	Gln	Gly 200	Leu	Ser	Ser	Pro	Val 205	Thr	Lys	Ser	
Phe	Asn 210	Arg	Gly	Glu	Сүз											
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atg	ggete	ggt d	atat	tatta	at to	etett	tctt	: gtt	gcta	accg	ctad	ccgat	gt q	gcact	ctcaa	60
gtco	caact	cg t	acaa	accaç	gg cg	getga	aagto	c gta	aaad	cccg	gaad	catct	gt 1	caaad	etetea	120
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ggad	caage	gee t	cgaa	atgga	at tç	ggcga	aaatt	: aac	ccaa	acta	acgo	gacat	ac t	caatt	tataat	240
gaaa	aaatt	ta a	agggo	caaaq	ge ta	acact	ccaco	c gto	gata	aat	caad	ectet	cac a	agctt	tatatg	300
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ggat	caat	at t	cgat	tact	g gg	ggtca	aaggo	c act	ctco	etca	cagt	cago	ctc a	ag		412
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Ser	Vol	Lvs	Leu	Cor	a	Luc	Ala	Ser	C1.v	-	The	Dl				
	vai	-1	20	Ser	сув	цуъ		25	Gry	Tyr	1111	рпе	Thr 30	Thr	Tyr	
Trp	Met	His 35	20 Trp	Val	Asn	Gln	Ala 40	25 Pro	Gly	Tyr Gln	Gly	Leu 45	Thr 30 Glu	Thr Trp	Tyr Ile	

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Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr 70 75 65 80 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Asn Tyr Val Gly Ser Ile Phe Asp Tyr Trp Gly Gln Gly Thr 100 105 110 Leu Leu Thr Val Ser Ser 115 <210> SEQ ID NO 184 <211> LENGTH: 992 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) <400> SEQUENCE: 184 ccagcacaaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg 60 gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg acggtgtcgt 120 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag 180 gactetacte ceteageage gtggtgaceg tgeeeteeag eagettggge acceagaeet 240 acatetgeaa egtgaateae aageeeagea acaeeaaggt ggacaagaga gttgageeea 300 aatettgtga caaaaeteae acatgteeae egtgeeeage acetgaaete etggggggae 360 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc cggacccctg 420 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt 480 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca 540 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg 600 agtacaagtg caaggtetee aacaaageee teecageeee categagaaa accateteea 660 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggaggaga 720 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg 780 $\verb|ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc||$ 840 tggactccga cggctccttc ttcctctata gcaagctcac cgtggacaag agcaggtggc 900 agcagggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc 960 agaagageet etecetgtee eegggtaaat ga 992 <210> SEQ ID NO 185 <211> LENGTH: 330 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1 or 2) <400> SEQUENCE: 185 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys 1 5 10 15 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr 25 20 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser 40 45 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser 50 55 60

Leu 65	Ser	Ser	Val	Val	Thr 70	Val	Pro	Ser	Ser	Ser 75	Leu	Gly	Thr	Gln	Thr 80
Tyr	Ile	Суз	Asn	Val 85	Asn	His	Lys	Pro	Ser 90	Asn	Thr	ГЛа	Val	Asp 95	Lys
Arg	Val	Glu	Pro 100	Гла	Ser	Суз	Asp	Lys 105	Thr	His	Thr	Суз	Pro 110	Pro	Cys
Pro	Ala	Pro 115	Glu	Leu	Leu	Gly	Gly 120	Pro	Ser	Val	Phe	Leu 125	Phe	Pro	Pro
Lys	Pro 130	Lys	Asp	Thr	Leu	Met 135	Ile	Ser	Arg	Thr	Pro 140	Glu	Val	Thr	Суз
Val 145	Val	Val	Asp	Val	Ser 150	His	Glu	Asp	Pro	Glu 155	Val	ГÀа	Phe	Asn	Trp 160
Tyr	Val	Asp	Gly	Val 165	Glu	Val	His	Asn	Ala 170	ГÀа	Thr	Lys	Pro	Arg 175	Glu
Glu	Gln	Tyr	Asn 180	Ser	Thr	Tyr	Arg	Val 185	Val	Ser	Val	Leu	Thr 190	Val	Leu
His	Gln	Asp 195	Trp	Leu	Asn	Gly	Lys 200	Glu	Tyr	ГÀа	Сүз	Lys 205	Val	Ser	Asn
Lys	Ala 210	Leu	Pro	Ala	Pro	Ile 215	Glu	Lys	Thr	Ile	Ser 220	Lys	Ala	Lys	Gly
Gln 225	Pro	Arg	Glu	Pro	Gln 230	Val	Tyr	Thr	Leu	Pro 235	Pro	Ser	Arg	Glu	Glu 240
Met	Thr	Lys	Asn	Gln 245	Val	Ser	Leu	Thr	Cys 250	Leu	Val	Lys	Gly	Phe 255	Tyr
Pro	Ser	Asp	Ile 260	Ala	Val	Glu	Trp	Glu 265	Ser	Asn	Gly	Gln	Pro 270	Glu	Asn
Asn	Tyr	Lys 275	Thr	Thr	Pro	Pro	Val 280	Leu	Aab	Ser	Aab	Gly 285	Ser	Phe	Phe
Leu	Tyr 290	Ser	Lys	Leu	Thr	Val 295	Aab	Lys	Ser	Arg	Trp 300	Gln	Gln	Gly	Asn
Val 305	Phe	Ser	Суз	Ser	Val 310	Met	His	Glu	Ala	Leu 315	His	Asn	His	Tyr	Thr 320
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tgca	aaago	cct (cagga	ataca	ac ti	tcad	caact	t tao	tgga	atgc	attę	gggt	caa 1	ccaa	geeece
gga	caago	gee 1	tcgaa	atgga	at to	ggcga	aaatt	z aad	cccaa	acta	acgo	gaca	tac 1	caatt	tataat
gaa	aaatt	ta a	agggo	caaaq	gc ta	acact	caco	c gto	cgata	aaat	саа	ecte	tac a	agctt	atatg
gaa	cttto	cat	ccct	gagat	cc aq	gaaga	ataca	a gco	cgtct	act	atto	gege	cag a	aaact	acgta
ggat	ccaat	at 1	cgat	taci	ad dá	ggtca	aaggo	c act	ceteo	ctca	cagt	cag	ctc a	ageca	agcaca
aag	ggeed	cat 🤇	cggt	cttco	20 CC	ctggo	cacco	e teo	eteca	aaga	gca	cete	tgg 🤅	gggca	acagcg
gcc	ctggg	get o	gcct	ggtca	aa go	gacta	actto	c cco	cgaad	ccgg	tga	cggt	gtc 🤉	gtgga	aactca

260

ggcé	geeet	ga (ccago	cggcó	gt go	cacad	cctto	c ccç	ggete	gtcc	taca	agtco	ctc a	aggad	ctctac	600
tcco	ctcag	gca 🤉	gcgto	ggtga	ac co	gtgco	ected	c ago	caget	tgg	gcad	cccaç	gac (ctaca	atctgc	660
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gaca	aaaad	etc a	acaca	atgto	cc ad	ccgt	geeca	a gca	accto	gaac	tcct	adaa	gaa (accgt	cagtc	780
ttco	ctctt	ccc (cccca	aaaa	cc ca	aagga	acaco	e eta	catga	atct	cccç	ggaco	ccc 1	tgago	gtcaca	840
tgco	gtggt	:gg 1	tggad	cgtga	ag co	cacga	aagad	c cct	gago	gtca	agtt	caad	etg o	gtaco	gtggac	900
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gaco	ggeta	ect 1	tette	cctct	ta ta	agcaa	ageto	c aco	gtgg	gaca	agag	gcago	gtg g	gcago	cagggg	1320
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Trp	Met	His 35	Trp	Val	Asn	Gln	Ala 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Ile	
Gly	Glu 50	Ile	Asn	Pro	Thr	Asn 55	Gly	His	Thr	Asn	Tyr 60	Asn	Glu	Lys	Phe	
Lys 65	Gly	Lys	Ala	Thr	Leu 70	Thr	Val	Asp	Lys	Ser 75	Thr	Ser	Thr	Ala	Tyr 80	
Met	Glu	Leu	Ser	Ser 85	Leu	Arg	Ser	Glu	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	Суз	
Ala	Arg	Asn	Tyr 100	Val	Gly	Ser	Ile	Phe 105	Aap	Tyr	Trp	Gly	Gln 110	Gly	Thr	
Leu	Leu	Thr 115	Val	Ser	Ser	Ala	Ser 120	Thr	Lys	Gly	Pro	Ser 125	Val	Phe	Pro	
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Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160	
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln	
Ser	Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser 185	Val	Val	Thr	Val	Pro 190	Ser	Ser	
Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser	

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Asn	Thr 210	ГЛа	Val	Asp	ГЛа	Arg 215	Val	Glu	Pro	ГЛа	Ser 220	Сүз	Asp	ГЛа	Thr	
His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240	
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg	
Thr	Pro	Glu	Val 260	Thr	Сүз	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro	
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala	
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val	
Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Гла	Glu	Tyr 320	
LÀa	Cys	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr	
Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu	
Pro	Pro	Ser 355	Arg	Glu	Glu	Met	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Thr	Сув	
Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser	
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400	
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser	
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	CÀa	Ser	Val	Met	His 430	Glu	Ala	
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ggad	aagg	jac t	cgaa	itgga	it ag	làcàs	aata	aat	ccca	icta	atgo	jacat	ac a	aatt	ataat	240
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gaad	tcag	jct c	cctc	cgat	c cg	jaaga	icact	gcc	gttt	att	atte	Itgcc	ag a	aact	atgta	360
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266

1380 1404

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Trp	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Ile
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Gln 65	Gly	Arg	Ala	Thr	Leu 70	Thr	Val	Asp	Lys	Ser 75	Thr	Ser	Thr	Ala	Tyr 80
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Ala	Arg	Asn	Tyr 100	Val	Gly	Ser	Ile	Phe 105	Asp	Tyr	Trp	Gly	Gln 110	Gly	Thr
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Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Сув	Asn	Val	Asn	His 205	Lys	Pro	Ser
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His 225	Thr	Суз	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	ГÀа	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg
Thr	Pro	Glu	Val 260	Thr	Сүз	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
Lys	Cys	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
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270

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Ser 65	Gly	Ser	Ala	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Val	Gln	Ala 80
Glu	Asp	Val	Ala	Asp 85	Tyr	His	Cys	Gly	Gln 90	Ser	Tyr	Asn	Tyr	Pro 95	Tyr
Thr	Phe	Gly	Gln 100	Gly	Thr	ГЛа	Leu	Glu 105	Ile	ГЛа	Arg	Thr	Val 110	Ala	Ala
Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly
Thr	Ala 130	Ser	Val	Val	Сүз	Leu 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Arg	Glu	Ala
Lys 145	Val	Gln	Trp	Гла	Val 150	Asp	Asn	Ala	Leu	Gln 155	Ser	Gly	Asn	Ser	Gln 160
Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser
Ser	Thr	Leu	Thr 180	Leu	Ser	Lys	Ala	Asp 185	Tyr	Glu	Lys	His	Lys 190	Val	Tyr
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Gly															
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Gly															
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278

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agaagageet etceetgtet eegggtaaat ga <210> SEO ID NO 208 <211> LENGTH: 330 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1 or 2) <400> SEQUENCE: 208 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys $\ensuremath{\mathsf{Pro}}$ Lys $\ensuremath{\mathsf{Asp}}$ Thr Leu Met Ile Ser $\ensuremath{\mathsf{Arg}}$ Thr $\ensuremath{\mathsf{Pro}}$ Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

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<211> LENGTH: 1404 <212> TYPE: DNA

<pre><212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <220> OTHER INFORMATION: Full Length Humanized Hu2B8 Heavy Chain (Hv5-51.1 Variable region)</pre>											
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tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc	180										
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Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Asn Tyr Val Gly Ser Ile Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys <210> SEQ ID NO 211

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:

286

2209

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Ser	Tyr	Lys	Gly	Thr 85	Val	Ser	Ile	Thr	Lys 90	Ser	Gly	Ile	Lys	Сув 95	Gln
Pro	Trp	Asn	Ser 100	Met	Ile	Pro	His	Glu 105	His	Ser	Phe	Leu	Pro 110	Ser	Ser
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Thr	Суа	Gln	Arg 180	Trp	Asp	Gln	Gln	Thr 185	Pro	His	Arg	His	Lys 190	Phe	Leu
Pro	Glu	Arg 195	Tyr	Pro	Asp	ГЛа	Gly 200	Phe	Asp	Asp	Asn	Tyr 205	Суз	Arg	Asn
Pro	Asp 210	Gly	Lys	Pro	Arg	Pro 215	Trp	Cys	Tyr	Thr	Leu 220	Asp	Pro	Asp	Thr
Thr 225	Trp	Glu	Tyr	Сүз	Ala 230	Ile	Lys	Thr	Cys	Ala 235	His	Ser	Ala	Val	Asn 240
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Asn	Phe 290	Lys	Суз	Lys	Asp	Leu 295	Arg	Glu	Asn	Tyr	Суз 300	Arg	Asn	Pro	Asp
Gly 305	Ala	Glu	Ser	Pro	Trp 310	Сүз	Phe	Thr	Thr	Asp 315	Pro	Asn	Ile	Arg	Val 320
Gly	Tyr	Cys	Ser	Gln 325	Ile	Pro	Lys	Cys	Asp 330	Val	Ser	Ser	Gly	Gln 335	Asp
Cys	Tyr	Arg	Gly 340	Asn	Gly	Lys	Asn	Tyr 345	Met	Gly	Asn	Leu	Ser 350	Lys	Thr
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Суз	Phe	Pro	Ser	Arg 485	Asp	Leu	Lys	Asp	Tyr 490	Glu	Ala	Trp	Leu	Gly 495	Ile
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Val	Ser	Gln 515	Leu	Val	Tyr	Gly	Pro 520	Glu	Gly	Ser	Asp	Leu 525	Val	Leu	Met
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Arg	Gly	Cys	Ala	Ile 645	Pro	Asn	Arg	Pro	Val 650	Ile	Phe	Val	Arg	Val 655	Ala
Tyr	Tyr	Ala	Lys 660	Trp	Ile	His	Lys	Val 665	Ile	Leu	Thr	Tyr	Lys 670	Leu	Сув
Gly	Arg	His 675	His	His	His	His	His 680								
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cato	gaatt	ta a	aaaq	gtcag	gc aa	aaaad	ctact	c ctt	acca	agg	aaga	accca	att a	actga	agatt
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Arg	Pro 210	Trp	Суз	Tyr	Thr	Leu 215	Asp	Pro	Asp	Thr	Pro 220	Trp	Glu	Tyr	Суз
Ala 225	Ile	Lys	Thr	Суа	Ala 230	His	Ser	Ala	Val	Asn 235	Glu	Thr	Asp	Val	Pro 240
Met	Glu	Thr	Thr	Glu 245	Суз	Ile	Gln	Gly	Gln 250	Gly	Glu	Gly	Tyr	Arg 255	Gly
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Gln	Tyr	Pro 275	His	Lys	His	Asp	Ile 280	Thr	Pro	Glu	Asn	Phe 285	Гла	Суз	Гла
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Tyr	Gly	Pro 515	Glu	Gly	Ser	Asp	Leu 520	Val	Leu	Leu	Lys	Leu 525	Ala	Arg	Pro
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Ile	His	Lys	Val 660	Ile	Leu	Thr	Tyr	Lys 665	Leu	Cys	Gly	Arg	His 670	His	His
His	His	His 675													

What is claimed is:

1. An isolated antibody that binds human hepatocyte growth factor (HGF), comprising:

- (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3}, wherein
 - (i) CDR_{H1} comprises the amino acid sequence of SEQ ID NO. 15,
 - (ii) CDR_{H2} comprises the amino acid sequence of SEQ ID NO. 205, and
 - (iii) CDR_{H3} comprises the amino acid sequence of SEQ $_{40}$ ID NO. 17, and
- (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein
 - (i) CDR_{L1} comprises the amino acid sequence of SEQ ID NO. 18,
 - (ii) CDR_{L2} comprises the amino acid sequence of SEQ ID NO. 206, and
 - (iii) CDR_{L3} comprises the amino acid sequence of SEQ ID NO. 20, or an antigen binding fragment of the antibody. 50

2. The antibody of claim 1, wherein the CDRs are interposed between human or humanized immunoglobulin framework regions.

3. The antibody of claim **1**, wherein the antibody is a monoclonal antibody.

4. The antibody of claim 1, wherein the antibody binds human hepatocyte growth factor with a k_d of 4.0×10^{-5} s⁻¹ or lower.

5. The antibody of claim 1, wherein the antibody binds human hepatocyte growth factor with a K_D of 20 pM or lower.

6. An isolated antibody that binds human hepatocyte growth factor (HGF) comprising an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO. 199, and an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO. 189, or an antigen binding fragment of the antibody.

7. An isolated antibody that binds human hepatocyte growth factor (HGF) comprising an immunoglobulin light chain sequence comprising the amino acid sequence of SEQ ID NO. 201, and an immunoglobulin heavy chain sequence comprising the amino acid sequence of SEQ ID NO. 191, or an antigen binding fragment of the antibody.

8. The antibody of claim **6**, wherein the antibody is a monoclonal antibody.

9. The antibody of claim **7**, wherein the antibody is a monoclonal antibody.

* * * * *