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WO-A2-03/094859
WO-A2-2005/051307
WO-A2-2006/084226
WO-A2-2007/030642
WO-A2-2007/075706
DALL'ACQUA WILLIAM F ET AL: "Modulation of the effector functions of a human IgG1 through engineering of its hinge region", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 177, no. 2, 15 July 2006 (2006-07-15) , pages 1129-1138, XP002497452, ISSN: 0022-1767
LANDEN CHARLES N JR ET AL: "Efficacy and antivascular effects of EphA2 reduction with an agonistic antibody in ovarian cancer.", JOURNAL OF THE NATIONAL CANCER INSTITUTE 1 NOV 2006 LNKD-PUBMED:17077358, vol. 98, no. 21, 1 November 2006 (2006-11-01), pages 1558-1570, XP002685564, ISSN: 1460-2105
DALL'ACQUA W F ET AL: "Antibody humanization by framework shuffling", METHODS : A COMPANION TO METHODS IN ENZYMOLOGY, ACADEMIC PRESS INC., NEW YORK, NY, US, vol. 36, no. 1, 1 May 2005 (2005-05-01), pages 43-60, XP027216562, ISSN: 1046-2023 [retrieved on 2005-04-19]
DAMSCHRODER ET AL: "Framework shuffling of antibodies to reduce immunogenicity and manipulate

Fortsættes ...

functional and biophysical properties", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 44, no. 11, 16 March 2007 (2007-03-16), pages 3049-3060, XP005938388, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2006.12.019

KIEWLICH D. ET AL.: 'Anti-EphA2 antibodies decrease EphA2 protein levels in murine CT26 colorectal and human MDA-231 breast tumors but do not inhibit tumor growth' NEOPLASIA vol. 8, no. 1, 2006, pages 18 - 30, XP002535474

CARLES-KINCH K. ET AL.: 'Antibody targeting of the EphA2 tyrosine kinase inhibits malignant cell behavior' CANCER RES. vol. 62, no. 10, 2002, pages 2840 - 2847, XP002904266

COFFMAN KT ET AL.: 'Differential EphA2 epitope display on normal versus malignant cells' CANCER RES. vol. 63, no. 22, 2003, pages 7907 - 7912, XP002993150

DESCRIPTION

Technical Field

[0001] The present invention relates to an antibody having an inhibitory activity against cell malignant transformation and/or tumor cell growth. More specifically, the present invention relates to an antibody against EPHA2 and a pharmaceutical composition comprising the antibody.

Background Art

[0002] EPHA2 is a receptor tyrosine kinase that has a molecular weight of 130 kDa and has a single transmembrane domain (Molecular and Cellular Biology, 1990, vol. 10, p. 6316-6324). EPHA2 has a ligand-binding domain and two fibronectin type 3 domains present in the N-terminal extracellular region and a tyrosine kinase domain and a sterile- α -motif (SAM) domain present in the C-terminal intracellular region.

[0003] GPI-anchored plasma membrane proteins ephrin-A1 to ephrin-A5 are known as EPHA2 ligands (Annual Review of Neuroscience, 1998, vol. 21, p. 309-345). The ligand binding to EPHA2 activates the tyrosine kinase domain and phosphorylates tyrosine residues present in the EPHA2 intracellular region, resulting in signal transduction within the cell. It has also been reported that EPHA2 bound with the ligand is internalized into the cell through endocytosis and is eventually degraded by a proteasome (Molecular Cancer Research, 2002, vol. 1, p. 79-87).

[0004] High expression of EPHA2 has been reported clinically in many cancers, particularly, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme (Cancer Research, 2001, vol. 61, p. 2301-2306; International Journal of Cancer, 2003, vol. 103, p. 657-663; The Prostate, 1999, vol. 41, p. 275-280; American Journal of Pathology, 2003, vol. 163, p. 2271-2276; Cancer Science, 2005, vol. 96, p. 42-47; Clinical Cancer Research, 2003, vol. 9, p. 613-618; Oncology Reports, 2004, vol. 11, p. 605-611; and Molecular Cancer Research, 2005, vol. 3, p. 541-551). It has also been reported that: for esophagus cancer, EPHA2 expression-positive patients tend to have a high frequency of regional lymph node metastasis, a large number of lymph node metastases, and a poor degree of tumor differentiation and a low five-year survival rate (International Journal of Cancer, 2003, vol. 103, p. 657-663); for non-small cell lung cancer, patients highly expressing EPHA2 tend to have a low disease-free survival rate and to have recurrence, particularly of brain metastasis (Clinical Cancer Research, 2003, vol. 9, p. 613-618); and for colon cancer, EPHA2 expression-positive patients tend to have liver metastasis, lymphatic vessel invasion, and lymph node metastasis, and many patients with high clinical stage are EPHA2 expression-positive patients (Oncology Reports, 2004, vol. 11, p. 605-611).

[0005] Moreover, it has been reported that by the introduction of EPHA2 genes into cells, non-cancer cells acquire cancer phenotypes such as anchorage-independent growth ability, tubular morphology-forming ability on the extracellular matrix, and in vivo tumor growth ability (Cancer Research, 2001, vol. 61, p. 2301-2306), and cancer cells have enhanced invasiveness through the extracellular matrix (Biochemical and Biophysical Research Communications, 2004, vol. 320, p. 1096-1102; and Oncogene, 2004, vol. 23, p. 1448-1456). In addition, it has been reported that: the invasiveness or anchorage-independent growth of cancer cells and in vivo tumor growth are inhibited by knockdown of EPHA2 expression using siRNA (Oncogene, 2004, vol. 23, p. 1448-1456; and Cancer Research, 2002, vol. 62, p. 2840-2847); and the invasiveness, anchorage-independent growth, and tubular morphology-forming ability of cancer cells are inhibited by activating EPHA2 using fusion proteins of its ligand ephrin-A1 and a human IgG Fc region and inducing EPHA2 degradation through endocytosis (Cancer Research, 2001, vol. 61, p. 2301-2306; Molecular Cancer Research, 2005, vol. 3, p. 541-551; and Biochemical and Biophysical Research Communications, 2004, vol. 320, p. 1096-1102).

[0006] On the other hand, EPHA2 has been reported to be expressed not only in cancer cells but also within tumors or in their surrounding blood vessels (Oncogene, 2000, vol. 19, p. 6043-6052). It has been reported that in mice, EPHA2 signals are involved in angiogenesis induced by ephrin-A1, and particularly, EPHA2 expressed in vascular endothelial cells is required for the tube formation or survival of the vascular endothelial cells (Journal of Cell Science, 2004, vol. 117, p. 2037-2049). It has also been reported that fusion proteins of an EPHA2 extracellular region and a human IgG Fc region inhibit angiogenesis in vivo and exhibit an antitumor effect (Oncogene, 2002, vol. 21, p. 7011-7026).

[0007] Monoclonal antibodies are useful not only as diagnostic drugs but also as therapeutic drugs. Monoclonal antibodies are actively used particularly in the field of cancer therapy, and monoclonal antibodies against receptor tyrosine kinases such as

HER2 and EGFR or against CD20 extracellular regions are used in cancer therapy and exhibit excellent effects (The New England Journal of Medicine, 2007, vol. 357, p. 39-51; Oncogene, 2007, vol. 26, p. 3661-3678; and Oncogene, 2007, vol. 26, p. 3603-3613). The mechanisms of action of the monoclonal antibodies used in cancer therapy include apoptosis induction and inhibition of growth signals. In addition, their immunoresponse-mediated action such as ADCC or CDC is also considered to play a very important role. In actuality, it has been reported that anti-HER2 antibodies (trastuzumab) or anti-CD20 antibodies (rituximab) exhibit a much weaker antitumor effect in xenografts of nude mice deficient in FcγRs necessary for ADCC induction than in nude mice that are not deficient in FcγRs, when these antibodies are administered thereto (Nature Medicine, 2000, vol. 6, p. 443-446). It has also been reported that anti-CD20 antibodies (rituximab) exhibit a weaker antitumor effect in mice depleted of complement by the administration of cobra venom than in mice that are not depleted of complement, when the antibody is administered thereto (Blood, 2004, vol. 103, p. 2738-2743).

[0008] For EPHA2, it has been reported that agonistic anti-EPHA2 monoclonal antibodies having an activity of inducing the phosphorylation of EPHA2 tyrosine residues and an activity of inducing EPHA2 degradation, as for the ligands, inhibit the anchorage-independent growth of a breast cancer cell line and the tubular morphology formation thereof on the extracellular matrix (Cancer Research, 2002, vol. 62, p. 2840-2847). It has also been reported that agonistic anti-EPHA2 monoclonal antibodies which recognize an epitope on EPHA2 displayed on cancer cells rather than non-cancer cells and have an activity of inducing the phosphorylation of EPHA2 tyrosine residues and an activity of inducing EPHA2 degradation exhibit an antitumor effect in vivo (Cancer Research, 2003, vol. 63, p. 7907-7912; and the pamphlet of WO 03/094859). On the other hand, Kiewich et al. have reported that their anti-EPHA2 monoclonal antibodies had an activity of inducing the phosphorylation of EPHA2 tyrosine residues and an activity of inducing EPHA2 degradation but did not exhibit an antitumor effect in vivo (Neoplasia, 2006, vol. 8, p. 18-30).

[0009] Moreover, the pamphlet of WO 2006/084226 discloses anti-EPHA2 monoclonal antibodies LUCA19, SG5, LUCA40, and SPL1 obtained by immunizing mice with cancer cells and discloses that, among these antibodies: LUCA19 and SG5 do not influence the phosphorylation of EPHA2 tyrosine residues; LUCA40 inhibits cancer cell growth in vitro; and LUCA19, SG5, and LUCA40 are internalized into cancer cells in the presence of anti-mouse antibody labeled with toxin (saporin). The document has also reported that LUCA40 and SPL1 exhibit an antitumor effect in vivo. However, the presence or absence of the agonistic activities of these antibodies remains to be clarified.

[0010] Despite these studies, an epitope for an anti-EPHA2 antibody that exhibits an antitumor effect in vivo is still unknown. No previous document has reported that a particular amino acid sequence in an EPHA2 extracellular region is useful as an epitope for a monoclonal antibody intended for cancer therapy.

[0011] Even antibodies against the same antigen differ in their properties depending on differences in epitopes or their sequences. Furthermore, due to this difference in their properties, the antibodies, when administered to humans, would clinically respond in different a manner with respect to drug effectiveness, the frequency of therapeutic response, side effects, the frequency of occurrence of drug resistance, etc.

[0012] Thus, a drug having clinically the best properties may also differ depending on the patient. In many cases, such properties are unknown until the drug is actually administered. Thus, it has been strongly required to develop a drug having a novel mechanism of action. It has also been strongly required to develop an antibody against EPHA2 having properties different from those of conventional antibodies.

Disclosure of the Invention

Problems to be Solved by the Invention

[0013] An object of the present invention is to provide an antibody against EPHA2.

[0014] A further object of the present invention is to provide a pharmaceutical composition and the like comprising an anti-EPHA2 antibody having a therapeutic effect on cancer.

[0015] A further object of the present invention is to provide a method for producing the antibody.

[0016] A further object of the present invention is to provide a method for inhibiting tumor growth using the antibody, etc.

Means for Solving the Problems

[0017] The present inventors have conducted diligent studies to attain the objects and consequently successfully obtained a novel anti-EPHA2 monoclonal antibody which has no activity of inducing the phosphorylation of EPHA2 tyrosine residues and has ADCC and CDC activities against EPHA2-expressing cancer cells. Furthermore, the present inventors have examined an epitope for this antibody and consequently found for the first time that an antibody that binds to a region comprising, of two fibronectin type 3 domains present in EPHA2, the c-terminal domain, has an excellent antitumor activity in vivo. Based on these findings, the present invention has been completed.

[0018] Specifically, the present invention comprises:

1. (1) An antibody which specifically binds to an EPHA2 polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing, wherein the antibody has no ability to phosphorylate EPHA2 tyrosine residues; and wherein the complementarity determining regions are selected from the following (i) to (iii):
 1. (i) the complementarity determining regions in the heavy chain variable region of the antibody have the amino acid sequences of either SEQ. ID NOS. 59, 61 and 63 or SEQ. ID NOS. 71, 73 and 75 and the complementarity determining regions in the light chain variable region of the antibody have the amino acid sequences of either SEQ. ID NOS. 65, 67 and 69 or SEQ. ID NOS. 77, 79 and 81;
 2. (ii) the antibody has the amino acid sequence of SEQ ID NOS. 109 as CDRH1, 111 as CDRH2 and 113 as CDRH3, and has the amino acid sequence of SEQ. ID NOS. 93 as CDRL1, 95 as CDRL2 and 97 as CDRL3; or
 3. (iii) the antibody has the amino acid sequence of SEQ ID NOS. 141 as CDRH1, 143 as CDRH2 and 145 as CDRH3 and has the amino acid sequence of SEQ. ID NOS. 125 as CDRL1, 127 as CDRL2 and 129 as CDRL3;
2. (2) The antibody according to (1) wherein the antibody is obtainable by humanizing an antibody consisting of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing; and
 2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;
3. (3) The antibody according to (2) wherein the antibody is obtainable by humanizing an antibody consisting of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing; and
 2. 2) a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing;
4. (4) The antibody according to (1), wherein the antibody exhibits an effect of decreasing an EPHA2 protein level;
5. (5) The antibody according to (1), wherein the antibody exhibits no effect of decreasing an EPHA2 protein level;
6. (6) The antibody according to any one of (1) to (5), wherein the antibody specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing;
7. (7) The antibody according to any one of (1) to (6), wherein the antibody inhibits the phosphorylation of EPHA2 tyrosine residues induced by an EPHA2 ligand;
8. (8) The antibody according to any one of (1) to (6), wherein the antibody does not inhibit EPHA2 ligand binding to EPHA2 but inhibits the phosphorylation of EPHA2 tyrosine residues induced by the ligand;
9. (9) The antibody according to (1), wherein the antibody has the amino acid sequences represented by SEQ ID NOS: 59, 61, and 63 in the sequence listing as the complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOS: 65, 67, and 69 in the sequence listing as complementarity determining regions in the light chain variable region;
10. (10) The antibody according to (9), wherein the antibody has the amino acid sequences represented by SEQ ID NOS: 109 as CDRH1, 111 as CDRH2 and 113 as CDRH3, and has the amino acid sequence represented by SEQ ID NOS: 93 as CDRL1, 95 as CDRL2 and 97 as CDRL3 in the sequence listing;
11. (11) The antibody according to (1) characterized by the following 1) and 2):
 1. 1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):

-FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4- (I)

wherein FRH1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH1 represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing; FRH2 represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH2 represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing; FRH3 represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH3 represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing; and FRH4 represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2. 2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):

-FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4- (II)

wherein FRL1 represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL1 represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing; FRL2 represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL2 represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL3 represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL3 represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing; and FRL4 represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds;

12. (12) The antibody according to (1) wherein the antibody has the amino acid sequences represented by SEQ ID NOS: 71, 73, and 75 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOS: 77, 79, and 81 in the sequence listing as complementarity determining regions in the light chain variable region;
13. (13) The antibody according to (12), wherein the antibody has the amino acid sequences represented by SEQ ID NOS: 141 as CDRH1, 143 as CDRH2 and 145 as CDRH3 and has the amino acid sequence represented by SEQ ID NOS: 125 as CDRL1, 127 as CDRL2 and 129 as CDRL3 in the sequence listing;
14. (14) The antibody according to (1) characterized by the following 1) and 2):
 1. 1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):

-FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4- (I)

wherein FRH1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH1 represents the amino acid sequence represented by SEQ ID NO: 71 in the sequence listing; FRH2 represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH2 represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing; FRH3 represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH3 represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing; and FRH4 represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2. 2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):

-FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4- (II)

wherein FRL1 represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL1 represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing; FRL2 represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL2 represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing; FRL3 represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL3 represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing; and FRL4 represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds;

15. (15) The antibody according to any one of (1), (4) to (14), characterized in that the antibody is a humanized antibody;
16. (16) The antibody according to any one of (1) to (14), characterized in that the antibody is a human antibody;
17. (17) The antibody according to any one of (1) to (14), characterized in that the antibody is an IgG antibody;
18. (18) The antibody according to any one of (1) to (8), characterized in that the antibody is any selected from Fab, F(ab')₂, Fv, scFv a diabody, a linear antibody, and a multispecific antibody;
19. (19) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by

- amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing; and
2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;
20. (20) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing.
 21. (21) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing; and
 2. 2) a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing;
 22. (22) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing;
 23. (23) An antibody produced by the hybridoma SH348-1 (FERM BP-10836);
 24. (24) An antibody produced by the hybridoma SH357-1 (FERM BP-10837);
 25. (25) An antibody obtained by humanizing an antibody according to any one of (20), (22), (23) or (24), wherein the antibody specifically binds to an EPHA2 polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing, and wherein the antibody has no ability to phosphorylate EPHA2 tyrosine residues;
 26. (26) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing; and
 2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;
 27. (27) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;
 28. (28) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing; and
 2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;
 29. (29) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ

- ID NO: 147 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;
30. (30) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 107 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 115 in the sequence listing; and
 2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 91 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 99 in the sequence listing;
 31. (31) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 107 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 91 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 115 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 99 in the sequence listing;
 32. (32) The antibody according to (1), wherein the antibody consists of the following 1) and 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 139 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 147 in the sequence listing; and
 2. 2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 123 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 131 in the sequence listing;
 33. (33) The antibody according to (1), wherein the antibody consists of the following 1) or 2):
 1. 1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 139 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 123 in the sequence listing; and
 2. 2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 147 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 131 in the sequence listing;
 34. (34) A Fab, F(ab')₂, Fv, scFv, a diabody, a linear antibody, or a multispecific antibody derivable from any antibodies according to any of (21), (22) or (25) to (29);
 35. (35) The mouse hybridoma SH348-1 (FERM BP-10836);
 36. (36) The mouse hybridoma SH357-1 (FERM BP-10837);
 37. (37) A pharmaceutical composition characterized by comprising at least one antibody selected from the antibodies according to (1) to (34);
 38. (38) A pharmaceutical composition for cancer treatment characterized by comprising at least one antibody selected from the antibodies according to (1) to (34);
 39. (39) An antibody or pharmaceutical composition according to (1) to (34), (37) or (38) for use in a method for inhibiting tumor growth in a mammal;
 40. (40) Use of an antibody or pharmaceutical composition according to (1) to (34), (37) or (38) for the manufacture of a medicament for inhibiting tumor growth in a mammal;
 41. (41) The antibody or pharmaceutical composition according to (39) or the use or pharmaceutical composition according to (40), characterized in that the tumor is a tumor expressing EPHA2;
 42. (42) A polynucleotide encoding an antibody or a polypeptide according to any one of (1) to (34);
 43. (43) A host cell transformed with a polynucleotide according to (42);
 44. (44) A method for producing an antibody using a host cell according to (43).

Advantages of the Invention

[0019] According to the present invention, a novel anti-EPHA2 monoclonal antibody has been successfully obtained, which has no activity of inducing the phosphorylation of EPHA2 tyrosine residues and has ADCC and CDC activities against EPHA2-expressing cancer cells. Furthermore, it has been found that the antibody has excellent antitumor activity in vivo.

[0020] Furthermore, a pharmaceutical composition for cancer treatment comprising the antibody has been provided.

Brief Description of the Drawings

[0021]

Figure 1 is a diagram showing Western blotting results showing the presence or absence of the activity of inducing the phosphorylation of EPHA2 tyrosine residues by an anti-EPHA2 antibody. Figure 1A) is a diagram showing the results obtained in the absence of a cross-linking antibody, wherein the upper bar shows the results for a 4G10 antibody, and the lower bar shows the results for an anti-EPHA2 antibody (D7). Figure 1B) is a diagram showing the results obtained in the presence of a cross-linking antibody, wherein the upper bar shows the results of a 4G10 antibody, and the lower bar shows the results of an anti-EPHA2 antibody (D7);

Figure 2 is a diagram showing Western blotting results showing the presence or absence of the activity of inducing a decrease in EPHA2 protein level by an anti-EPHA2 antibody. Figure 2A) is a diagram showing the results obtained in the absence of a cross-linking antibody, wherein the upper bar shows the results for an anti-EPHA2 antibody (D7), and the lower bar shows the results for an anti- β -actin antibody. Figure 2B) is a diagram showing the results obtained in the presence of a cross-linking antibody, wherein the upper bar shows the results for an anti-EPHA2 antibody (D7), and the lower bar shows the results for an anti- β -actin antibody;

Figure 3 is a diagram showing the presence or absence of the ADCC activity of an anti-EPHA2 antibody against various cell lines. In the diagram, "****" represents $P < 0.01$, and "*****" represents $P < 0.001$. Figure 3A) is a diagram showing the ADCC activity against MDA-MB-231 cells. Figure 3B) is a diagram showing the ADCC activity against A549 cells. Figure 3C) is a diagram showing the ADCC activity against PC-3 cells;

Figure 4 is a diagram showing the presence or absence of the CDC activity of an anti-EPHA2 antibody against various cells. In the diagram, "*****" represents $P < 0.001$. Figure 4A) is a diagram showing the CDC activity of SH348-1 against MDA-MB-231 cells. Figure 4B) is a diagram showing the CDC activity of SH348-1 against A549 cells. Figure 4C) is a diagram showing the CDC activity of SH348-1 against PC-3 cells. Figure 4D) is a diagram showing the CDC activity of SH357-1 against MDA-MB-231 cells. Figure 4E) is a diagram showing the CDC activity of SH357-1 against A549 cells. Figure 4F) is a diagram showing the CDC activity of SH357-1 against PC-3 cells;

Figure 5A) is a diagram showing EPHA2 domain structure prediction and the positions, in EPHA2, of EPHA2-ECD, FNIII-NC, FNIII-N, and FNIII-C which are peptides for epitope determination. In the diagram, Ligand-BD represents a ligand-binding domain, FN3 represents a fibronectin type 3 domain, TM represents a transmembrane region, Trk kinase represents a tyrosine kinase domain, and SAM represents a SAM domain;

Figure 5B) is a diagram showing the presence or absence of the binding activity of an anti-EPHA2 antibody for EPHA2-ECD, FNIII-NC, FNIII-N, and FNIII-C;

Figure 6A) is a diagram showing the antitumor activity of SH348-1 against MDA-MB-231 cell-transplanted mice;

Figure 6B) is a diagram showing the antitumor activity of SH357-1 against MDA-MB-231 cell-transplanted mice. In the diagram, the error bar represents a standard error ($n = 9$);

Figure 7A) is a diagram showing the binding activity of SH348-1 to an EPHA2 extracellular region polypeptide;

Figure 7B) is a diagram showing the binding activity of SH357-1 to an EPHA2 extracellular region polypeptide;

Figure 7C) is a diagram showing the binding activity of Ab96-1 to an EPHA2 extracellular region polypeptide;

Figure 8 is a diagram showing the ligand binding inhibitory activities of SH348-1, SH357-1, and Ab96-1;

Figure 9 is a diagram showing that SH348-1 and SH357-1 have an activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues;

Figure 10 is a diagram showing the outline of deletion mutants of EPHA2 for epitope identification;

Figure 11A) is a diagram showing the reactivity of SH348-1 to deletion mutants of EPHA2;

Figure 11B) is a diagram showing the detection of the deletion mutants of EPHA2 on a PVDF membrane in Figure 11A);

Figure 11C) is a diagram showing the reactivity of SH357-1 to deletion mutants of EPHA2;

Figure 11D) is a diagram showing the detection of the deletion mutants of EPHA2 on a PVDF membrane in Figure 11C);

Figure 12A) is a diagram showing the binding activity of hSH348-1-T1 to an EPHA2 extracellular region polypeptide;

Figure 12B) is a diagram showing the binding activity of hSH348-1-T3 to an EPHA2 extracellular region polypeptide;

Figure 12C) is a diagram showing the binding activity of hSH357-1-T1 to an EPHA2 extracellular region polypeptide;

Figure 12D) is a diagram showing the binding activity of hSH357-1-T3 to an EPHA2 extracellular region polypeptide;

Figure 13A) is a diagram showing the competitive inhibitory activities of hSH348-1-T1 and hSH348-1-T3 against the antigen binding of SH348-1;

Figure 13B) is a diagram showing the competitive inhibitory activities of hSH357-1-T1 and hSH357-1-T3 against the antigen binding of SH357-1;

Figure 14A) is a diagram showing the activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues by hSH348-1-T1 or hSH348-1-T3; and

Figure 14B) is a diagram showing the activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues by hSH357-1-T1 or hSH357-1-T3.

Best Mode for Carrying Out the Invention

1. Definitions

[0022] In the present specification, the terms "cancer" and "tumor" are used in the same sense.

[0023] In the present specification, the term "gene" is meant to encompass not only DNA but also mRNA thereof, cDNA, and crRNA thereof. Thus, the term "EPHA2 gene" in the present invention encompasses EPHA2 DNA, mRNA, cDNA, and crRNA.

[0024] In the present specification, the term "polynucleotide" is used in the same sense as a nucleic acid and also encompasses DNA, RNA, probes, oligonucleotides, and primers.

[0025] In the present specification, the terms "polypeptide" and "protein" are used without being differentiated therebetween.

[0026] In the present specification, the term "cell" also encompasses cells in individual animal and cultured cells.

[0027] In the present specification, the term "cell malignant transformation" means that cells exhibit abnormal growth, for example, lose sensitivity to contact inhibition or exhibit anchorage-independent growth.

[0028] Cells exhibiting such abnormal growth are referred to as "cancer cells".

[0029] In the present specification, a protein having equivalent functions to the cell malignant transformation activity and/or cell growth activity or the like of EPHA2 is also referred to as EPHA2.

[0030] In the present specification, the term "phosphorylation of tyrosine residues" means that tyrosine residues contained in the amino acid sequence of a peptide are phosphorylated. Whether or not tyrosine residues are phosphorylated can be examined, for example, based on the affinity of the peptide for an anti-phosphotyrosine antibody (e.g., Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (manufactured by Millipore (Upstate), #16-184)). The tyrosine residues can be determined to be phosphorylated, when the peptide binds to the antibody.

[0031] In the present specification, the term "ability to phosphorylate EPHA2 tyrosine residues" refers to an ability to phosphorylate tyrosine residues in the amino acid sequence of EPHA2. Whether or not an antibody has the ability to phosphorylate EPHA2 tyrosine residues can be determined, for example, by incubating the antibody and EPHA2 and then examining the presence or absence of the affinity of the EPHA2 for an anti-phosphotyrosine antibody.

[0032] In the present specification, the phrase "decreasing an EPHA2 protein expression level" means that an EPHA2 protein level is decreased. Whether or not an antibody has an effect of decreasing an EPHA2 protein level can be examined, for example, by incubating the antibody and EPHA2 and then quantifying the EPHA2 level.

[0033] In the present specification, the term "EPHA2 ligand" refers to a substance capable of serving as an EPHA2 ligand. Specific examples thereof can include GPI-anchored plasma membrane proteins ephrin-A1 to ephrin-A5 (Annual Review of Neuroscience, 1998, vol. 21, p. 309-345).

[0034] In the present specification, the term "cytotoxicity" refers to any pathologic change in cells and refers not only to direct injury but also to any structural or functional damage to cells such as DNA cleavage, dimerization of bases, chromosomal breakage, damage of mitotic apparatus, and decrease in various enzyme activities.

[0035] In the present specification, the term "cytotoxic activity" refers to an activity that causes the cytotoxicity.

[0036] In the present specification, ADCC is synonymous with antibody-dependent cellular cytotoxicity and refers to a reaction through which Fcγ receptor-bearing cells adhere via the Fcγ receptors to the Fc portions of antibodies bound with surface antigens in target cells and kill the target cells. An ADCC activity is also referred to as an antibody-dependent cytotoxic activity and refers to an activity that causes the reaction. The ADCC activity can be measured by methods usually performed by those skilled in the art and can be measured, for example, according to a method described in paragraph 3)-2 of Example 3 in the present specification.

[0037] In the present specification, the term "CDC" is synonymous with complement-dependent cytotoxicity. A CDC activity refers to an activity that causes complement-dependent cytotoxicity. The CDC activity can be measured by methods usually performed by those skilled in the art and can be measured, for example, according to a method described in paragraph 3)-3 of Example 3 in the present specification.

[0038] In the present specification, the phrase "having an antitumor activity in vivo" means having the activity of inhibiting or reducing tumor growth in tumor-bearing animal individuals. Whether or not an anti-EPHA2 antibody "has an antitumor activity in vivo" can be examined by methods usually performed by those skilled in the art and can also be examined, for example, according to the following method: an appropriate dose of the anti-EPHA2 antibody is intraperitoneally administered as a test substance to tumor cell (e.g., MDA-MB-231 cell)-subcutaneously transplanted nude mice (e.g., BALB/cAJc1-nu/nu; obtained from CLEA Japan, Inc.), and the time-dependent change in tumor volume is compared between the nude mice and anti-EPHA2 antibody-unadministered controls. The anti-EPHA2 antibody as a test substance can be determined to "have an antitumor activity in vivo", when the tumor volume is significantly smaller in the mice than in the controls.

[0039] Each of the heavy and light chains of an antibody molecule is known to have three complementarity determining regions (CDRs). In the present specification, the complementarity determining regions of an antibody are represented by CDRH₁, CDRH₂, and CDRH₃ for the complementarity determining regions of the heavy chain and by CDRL₁, CDRL₂, and CDRL₃ for the complementarity determining regions of the light chain.

[0040] In the present specification, the term "epitope" means an EPHA2 partial peptide having antigenicity and/or immunogenicity in vivo in animals, preferably, mammals, more preferably mice or humans. The epitope as an EPHA2 partial peptide having antigenicity can be determined by methods well known by those skilled in the art, such as by an immunoassay, and can be determined, for example, according to the following method in which various partial structures of EPHA2 are prepared. For the preparation of the partial structures, oligopeptide synthesis techniques known in the art can be used. For example, a series of sequentially shorter polypeptides of appropriate lengths from the C- or N-terminus of EPHA2 is prepared using a gene recombination technique well known by those skilled in the art. Then, the reactivity of the antibody to these polypeptides is studied. A recognition site is roughly determined, and shorter peptides are then synthesized. The reactivity to these peptides can be studied to thereby determine the epitope.

1. Regarding EPHA2

(1) EPHA2 gene

[0041] The nucleotide sequence of the EPHA2 gene and the amino acid sequence thereof are recorded as EPH receptor A2 in GenBank (Accession Nos: NM_004431 and NP_004422, respectively). Moreover, the nucleotide sequence of an open reading frame (ORF) in the EPHA2 gene is described in SEQ ID NO: 1 in the sequence listing. The amino acid sequence thereof is described in SEQ ID NO: 2 in the sequence listing.

[0042] In this context, EPHA2 also encompasses proteins which consist of an amino acid sequence derived from the EPHA2 amino acid sequence by the substitution, deletion, or addition of one or more amino acids and have an equivalent biological activity to that of this enzyme.

(2) Cancer site-specific expression of EPHA2 gene

[0043] The EPHA2 gene has been reported to be highly expressed in many cancers, particularly, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme.

[0044] Specifically, the expression level of EPHA2 in each cell and/or each tissue can be measured to thereby determine the state of malignant transformation and/or cancer cell growth that can be attributed to EPHA2 over-expression in test subjects.

[0045] Moreover, a substance that inhibits the expression level and/or activity of EPHA2 has an activity of inhibiting cell malignant transformation and/or cancer cell growth attributed to EPHA2.

[0046] Thus, test substances are contacted with EPHA2-expressing cells, and a substance that inhibits the expression level and/or activity of EPHA2 can be selected to thereby screen for an antitumor substance.

[0047] In this context, siRNA against EPHA2 inhibits EPHA2 expression and can be used as an antitumor agent. The siRNA against EPHA2 can be produced by: designing, based on the nucleotide sequence of EPHA2 mRNA, RNA consisting of a partial sequence of EPHA2 mRNA (sense RNA) and RNA consisting of a nucleotide sequence complementary to the nucleotide sequence of the RNA (antisense RNA); synthesizing the RNAs by a chemical synthesis method known per se in the art; and hybridizing both the obtained RNAs. It is preferred that a sequence of one or more nucleotides called an overhang sequence should be bound to the 3'-end of each of the sense and antisense RNAs constituting the siRNA. The overhang sequence is not particularly limited as long as it protects the RNA from nuclease. Any sequence of preferably 1 to 10, more preferably 1 to 4, even more preferably 2 nucleotides can be used.

2. Antibody against EPHA2

(1) Preparation of antigen

[0048] Examples of antigens for obtaining the antibody of the present invention against EPHA2 can include a full-length polypeptide of EPHA2 and partial polypeptides thereof and can more specifically include a full-length polypeptide of EPHA2 and preferably an EPHA2 extracellular region polypeptide (consisting of an amino acid sequence represented by amino acid Nos. 1 to 534 of SEQ ID NO: 8 in the sequence listing), more preferably a partial polypeptide of the EPHA2 extracellular region polypeptide comprising an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing, even more preferably a partial polypeptide of the EPHA2 extracellular region polypeptide comprising an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing, and derivatives obtained by adding an arbitrary amino acid sequence or a carrier to these sequences. Further examples thereof can include polypeptides consisting of consecutive partial amino acid sequences of at least 6 amino acids and derivatives obtained by adding an arbitrary amino acid sequence or a carrier to these sequences.

[0049] In this context, the EPHA2 full-length polypeptide or the partial polypeptides thereof used as an antigen can be obtained by causing the EPHA2 gene or genes of the partial polypeptides to be expressed in host cells by genetic engineering.

[0050] EPHA2 can be directly purified, for use, from human tumor tissues or tumor cells. Moreover, the EPHA2 full-length polypeptide or the partial polypeptides thereof can be synthesized in vitro or obtained by causing host cells by genetic engineering to produce the polypeptide.

[0051] In the genetic engineering, specifically, genes encoding EPHA2 or partial polypeptides thereof are incorporated into vectors capable of expressing the EPHA2 or partial polypeptides thereof, and the EPHA2 or partial polypeptides thereof can then be synthesized in a solution containing enzymes, substrates, and energy substances necessary for transcription and translation. Alternatively, host cells of other prokaryotes or eukaryotes can be transformed therewith and caused to express the EPHA2 or partial polypeptides thereof to obtain the desired protein.

[0052] cDNA of the partial polypeptide of EPHA2 can be obtained, for example, by a so-called polymerase chain reaction (hereinafter, referred to as "PCR") method in which PCR (see Saiki, R.K., et al. Science (1988) 239, p. 487-489) is performed using EPHA2-expressing cDNA libraries as templates and primers specifically amplifying EPHA2 cDNA or DNA encoding the partial polypeptide.

[0053] Examples of in vitro polypeptide synthesis include, but are not limited to, the Rapid Translation System (RTS) manufactured by Roche Diagnostics Corp.

[0054] Examples of the host prokaryotic cells include Escherichia coli and Bacillus subtilis. To transform these host cells with the gene of interest, the host cells are transformed with plasmid vectors comprising a replicon, i.e., a replication origin, and a regulatory sequence derived from a species compatible with the hosts. Moreover, it is preferred that the vectors should have a sequence that can impart phenotypic character (phenotype) selectivity to the transformed cells.

[0055] The host eukaryotic cells encompass cells of vertebrates, insects, yeast, and the like. For example, monkey COS cells (Gluzman, Y. Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L.A., Proc. Natl. Acad. Sci. USA (1980) 77, p. 4126-4220) of Chinese hamster ovarian cells (CHO cells, ATCC CCL-61) are often used as the vertebrate cells, though the vertebrate cells are not limited thereto.

[0056] The transformants thus obtained can be cultured according to standard methods and are able within the culture to intracellularly or extracellularly produce the polypeptide of interest.

[0057] A medium used in the culture can be selected appropriately according to the adopted host cells from among various media routinely used. For Escherichia coli, for example, an LB medium optionally supplemented with an antibiotic (e.g., ampicillin) or IPTG can be used.

[0058] The recombinant protein intracellularly or extracellularly produced by the transformants in the culture can be separated and purified by various separation procedures known in the art using the physical properties, chemical properties, or the like of the protein.

[0059] The procedures can be exemplified specifically by treatment with usual protein precipitants, ultrafiltration, various liquid chromatography techniques such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion-exchange chromatography, affinity chromatography, and high-performance liquid chromatography (HPLC), dialysis, and combinations thereof.

[0060] Moreover, the recombinant protein to be expressed can be linked to 6 histidine residues to thereby efficiently purify the resulting protein on a nickel affinity column.

[0061] By combining these methods, the polypeptide of interest can be produced easily in large amounts with high yields and high purity.

[0062] The antibody of the present invention can be obtained by immunizing animals with the antigen according to a standard method and collecting antibodies produced in vivo, followed by purification.

[0063] Moreover, antibody-producing cells that produce the antibody against EPHA2 are fused with myeloma cells according to a method known in the art (e.g., Kohler and Milstein, Nature (1975) 256, p. 495-497; and Kennet, R. ed., Monoclonal Antibodies, p. 365-367, Plenum Press, N.Y. (1980)) to thereby establish hybridomas, from which monoclonal antibodies can also be obtained.

(2) Production of anti-EPHA2 monoclonal antibody

[0064] Examples of antibodies which specifically bind to EPHA2 can include monoclonal antibodies which specifically bind to EPHA2. A method for obtaining the antibodies is as described below.

[0065] For monoclonal antibody production, the following process is generally required:

1. (a) the step of purifying biopolymers used as antigens,
2. (b) the step of immunizing animals with the antigens through injection, then collecting blood from the animals, assaying the antibody titer thereof to determine the timing of splenectomy, and then preparing antibody-producing cells,
3. (c) the step of preparing myeloma cells (hereinafter, referred to as "myelomas"),
4. (d) the step of performing cell fusion between the antibody-producing cells and the myelomas,
5. (e) the step of selecting a hybridoma group that produces the antibody of interest,
6. (f) the step of dividing into single cell clones (cloning),
7. (g) the step of culturing the hybridomas for producing monoclonal antibodies in large amounts or raising hybridoma-transplanted animals, according to circumstances,
8. (h) the step of studying the bioactivities and binding specificities of the monoclonal antibodies thus produced or assaying properties as labeling reagents, etc.

[0066] Hereinafter, the method for preparing monoclonal antibodies will be described in detail in line with these steps, though the method for preparing antibodies is not limited thereto. For example, antibody-producing cells other than splenic cells and myelomas can also be used.

(a) Purification of antigens

[0067] EPHA2 or partial polypeptides thereof prepared by the method as described above can be used as antigens.

[0068] Moreover, partial peptides of the protein, which are chemically synthesized using membrane fractions prepared from EPHA2-expressing recombinant somatic cells or the EPHA2-expressing recombinant somatic cells themselves according to a method well known by those skilled in the art, can also be used as antigens.

(b) Preparation of antibody-producing cells

[0069] The antigens obtained in step (a) are mixed with adjuvants well known by those skilled in the art, for example, complete or incomplete Freund's adjuvants, or other auxiliaries such as potassium aluminum sulfate, and experimental animals are immunized with these immunogens. Animals used in hybridoma preparation methods known in the art can be used as the experimental animals without problems. Specifically, for example, mice, rats, goats, sheep, cow, and horses can be used. However, mice or rats are preferably used as the animals to be immunized, from the viewpoint of the easy availability of myeloma cells to be fused with the extracted antibody-producing cells, etc.

[0070] Moreover, the lineages of the mice and rats actually used are not particularly limited. For example, mouse lineages such as A, AKR, BALB/c, BDP, BA, CE, C3H, 57BL, C57BR, C57L, DBA, FL, HTH, HT1, LP, NZB, NZW, RF, R III, SJL, SWR, WB, and 129 and rat lineages such as Low, Lewis, Sprague, Dawley, ACI, BN, and Fischer can be used.

[0071] These mice and rats can be obtained from, for example, experimental animal growers/distributors such as CLEA Japan, Inc. and Charles River Laboratories Japan, Inc.

[0072] Of these lineages, the mouse BALB/c lineage and the rat Low lineage are particularly preferable as the animals to be immunized, in consideration of fusion compatibility with myeloma cells as described later.

[0073] Moreover, mice having a reduced biological mechanism for autoantibody removal, i.e., autoimmune disease mice, are also preferably used in consideration of the antigenic homology between humans and mice.

[0074] These mice or rats are preferably 5 to 12 weeks old, more preferably 6 to 8 weeks old, at the time of immunization.

[0075] For the immunization of the animals with EPHA2 or the recombinant antigens thereof, methods known in the art described in detail in, for example, Weir, D.M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987), and Kabat, E.A. and Mayer, M.M., Experimental Immunochemistry, Charles C Thomas Publisher Springfield, Illinois (1964) can be used.

[0076] Of these immunization methods, a method preferable is specifically illustrated as described below.

[0077] Specifically, the membrane protein fractions used as antigens or antigen-expressing cells are first administered intradermally or intraperitoneally to the animals.

[0078] However, the combined use of both of the administration routes is preferable for enhancing immunization efficiency. Immunization efficiency can be enhanced particularly by performing intradermal administration in early immunizations and performing intraperitoneal administration in later immunizations or only in the last immunization.

[0079] The administration schedule of the antigens differs depending on the type of animals to be immunized, the individual differences thereof, etc. The antigens are generally administered at 3 to 6 doses preferably at 2-to 6-week intervals, more preferably at 3 to 4 doses at 2- to 4-week intervals.

[0080] Moreover, the dose of the antigens differs depending on the type of animals, the individual differences thereof, etc., and is generally of the order of 0.05 to 5 mg, preferably 0.1 to 0.5 mg.

[0081] A booster is performed 1 to 6 weeks later, preferably 2 to 4 weeks later, more preferably 2 to 3 weeks later, from such antigen administration.

[0082] In this context, the dose of the antigens in the booster differs depending on the type of animals, the size thereof, etc., and is generally of the order of 0.05 to 5 mg, preferably 0.1 to 0.5 mg, more preferably 0.1 to 0.2 mg, for example, for mice.

[0083] 1 to 10 days later, preferably 2 to 5 days later, more preferably 2 to 3 days later, from the booster, splenic cells or lymphocytes containing antibody-producing cells are aseptically extracted from the animals to be immunized.

[0084] In this procedure, their antibody titers are measured, and animals having a sufficiently increased antibody titer can be used as sources of antibody-producing cells to thereby enhance the efficiency of the subsequent procedures.

[0085] Examples of methods for measuring the antibody titers used here can include, but not limited to, RIA and ELISA.

[0086] The antibody titer measurement can be performed by procedures as described below, for example, according to ELISA.

[0087] First, the purified or partially purified antigens are adsorbed onto the surface of a solid phase such as 96-well plates for ELISA. Furthermore, antigen-unadsorbed solid phase surface is covered with proteins unrelated to the antigens, for example, bovine serum albumin (hereinafter, referred to as "BSA"). The surfaces are washed and then contacted with serially diluted samples (e.g., mouse serum) as primary antibodies such that the antibodies in the samples are bound to the antigens.

[0088] Furthermore, enzyme-labeled antibodies against the mouse antibodies are added thereto as secondary antibodies such that the secondary antibodies are bound to the mouse antibodies. After washing, substrates for the enzyme are added thereto, and, for example, the change in absorbance caused by color development based on substrate degradation is measured to thereby calculate antibody titers.

[0089] The antibody-producing cells can be separated from these spleen cells or lymphocytes according to methods known in the art (e.g., Kohler et al., Nature (1975) 256, p. 495; Kohler et al., Eur. J. Immunol. (1977) 6, p. 511; Milstein et al., Nature (1977), 266, p. 550; and Walsh, Nature, (1977) 266, p. 495).

[0090] For example, for the spleen cells, a general method can be adopted, which involves cutting the cells into strips, filtering them through a stainless mesh, and then separating the antibody-producing cells therefrom by floating in Eagle's minimal essential medium (MEM).

(C) Preparation of myeloma cells (hereinafter, referred to as "myelomas")

[0091] Myeloma cells used in cell fusion are not particularly limited and can be selected appropriately, for use, from cell strains known in the art. However, HGPRT (hypoxanthine-guanine phosphoribosyl transferase)-deficient strains for which selection methods have been established are preferably used in consideration of convenient hybridoma selection from fused cells.

[0092] Specific examples thereof include: mouse-derived X63-Ag8 (X63), NS1-ANS/1 (NS1), P3X63-Ag8. U1(P3U1), X63-Ag8.653 (X63.653), SP2/0-Ag14 (SP2/0), MPC11-45.6TG1.7 (45.6TG), FO, S149/5XXO, and BU.1; rat-derived 210.RSY3.Ag.1.2.3 (Y3); and human-derived U266AR (SKO-007), GM1500.GTG-A12 (GM1500), UC729-6, LICR-LON-HMy2 (HMy2), and 8226AR/NIP4-1 (NP41).

[0093] These HGPRT-deficient strains can be obtained from, for example, American Type Culture Collection (ATCC).

[0094] These cell strains are subcultured in an appropriate medium, for example, an 8-azaguanine medium [RPMI-1640 medium supplemented with glutamine, 2-mercaptoethanol, gentamicin, and fetal bovine serum (hereinafter, referred to as "FBS") and further supplemented with 8-azaguanine], an Iscove's Modified Dulbecco's Medium (hereinafter, referred to as "IMDM"), or a Dulbecco's Modified Eagle Medium (hereinafter, referred to as "DMEM") and subcultured in a normal medium [e.g., an ASF104 medium (manufactured by Ajinomoto Co., Inc.) containing 10% FBS] 3 to 4 days before cell fusion. On the day of fusion, 2×10^7 or more cells are secured.

(d) Cell fusion

[0095] Fusion between the antibody-producing cells and the myeloma cells can be performed appropriately under conditions that do not excessively reduce the cell survival rates, according to methods known in the art (e.g., Weir, D.M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987); and Kabat, E.A. and Mayer, M.M., Experimental Immunochemistry, Charles C Thomas Publisher Springfield, Illinois (1964)).

[0096] For example, a chemical method which involves mixing the antibody-producing cells and the myeloma cells in a high-concentration polymer (e.g., polyethylene glycol) solution and a physical method which uses electric stimulations can be used as such methods.

[0097] Of these methods, the chemical method is specifically exemplified as described below.

[0098] Specifically, when polyethylene glycol is used as a polymer in the high-concentration polymer solution, the antibody-producing cells and the myeloma cells are mixed in a solution of polyethylene glycol having a molecular weight of 1500 to 6000, preferably 2000 to 4000, at a temperature of 30 to 40°C, preferably 35 to 38°C, for 1 to 10 minutes, preferably 5 to 8 minutes.

(e) Selection of hybridoma group

[0099] A method for selecting the hybridomas obtained by the cell fusion is not particularly limited, and a HAT (hypoxanthine-aminopterin-thymidine) selection method (Kohler et al., Nature (1975) 256, p. 495; and Milstein et al., Nature (1977) 266, p. 550) is usually used.

[0100] This method is effective for obtaining hybridomas using HGPRT-deficient myeloma cell strains that cannot survive in aminopterin.

[0101] Specifically, unfused cells and the hybridomas can be cultured in a HAT medium to thereby cause only aminopterin-resistant hybridomas to remain and grow.

(f) Dividing into single cell clones (cloning)

[0102] For example, methods known in the art, such as methylcellulose, soft agarose, and limiting dilution methods can be used as methods for cloning the hybridomas (see e.g., Barbara, B.M. and Stanley, M.S.: Selected Methods in Cellular Immunology, W.H. Freeman and Company, San Francisco (1980)). Of these methods, the limiting dilution method is particularly preferable.

[0103] In this method, feeders such as rat fetus-derived fibroblast strains or normal mouse splenic, thymus, or ascites cells are inoculated onto a microplate.

[0104] On the other hand, the hybridomas are diluted to 0.2 to 0.5 individuals/0.2 ml in advance in a medium. This solution containing the diluted hybridomas floating therein is added at a concentration of 0.1 ml/well, and the hybridomas can be continuously cultured for approximately 2 weeks while approximately 1/3 of the medium is replaced with a new one at regular intervals (e.g., 3-day intervals), to thereby grow hybridoma clones.

[0105] For wells having an observable antibody titer, for example, cloning by the limiting dilution method is repeated 2 to 4 times, and clones whose antibody titer is stably observed can be selected as anti-EPHA2 monoclonal antibody-producing hybridoma strains.

[0106] Examples of the hybridoma strains thus cloned can include hybridoma SH348-1 and hybridoma SH357-1. Hybridoma SH348-1 and hybridoma SH357-1 have been deposited on Jun. 8, 2007 with International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology, (address: Tsukuba Central 6, Higashi 1-1-1, Tsukuba, Ibaraki, Japan). Hybridoma SH348-1 has been designated as SH348-1 with Accession No. FERM BP-10836, and hybridoma SH357-1 has been designated as SH357-1 with Accession No. FERM BP-10837.

[0107] In the present specification, an antibody produced by hybridoma SH348-1 is referred to as "SH348-1", and an antibody produced by hybridoma SH357-1 is referred to as "SH357-1".

(g) Preparation of monoclonal antibodies by hybridoma culture

[0108] The hybridomas thus selected can be cultured to thereby efficiently obtain monoclonal antibodies. Prior to the culture, it is preferred that hybridomas producing the monoclonal antibody of interest should be screened.

[0109] For this screening, methods known per se in the art can be adopted.

[0110] The antibody titer measurement can be performed, for example, by ELISA as described in paragraph (b).

[0111] The hybridomas obtained by the method as described above can be cryopreserved in liquid nitrogen or in a freezer at -80°C or lower.

[0112] Moreover, the completely cloned hybridomas can be passaged several times in a HT medium (HAT medium except for aminopterin) and then cultured in a normal medium changed therefrom.

[0113] Large-scale culture is performed by rotational culture using large culture bottles or spinner culture.

[0114] A supernatant obtained in this large-scale culture can be purified according to methods well known by those skilled in the art, such as gel filtration, to obtain monoclonal antibodies which specifically bind to the protein.

[0115] Moreover, the hybridomas can be intraperitoneally injected to mice of the same lineage thereas (e.g., BALB/c) or Nu/Nu mice and grown to obtain ascites containing the monoclonal antibody of the present invention in large amounts.

[0116] For the intraperitoneal administration, mineral oil such as 2,6,10,14-tetramethyl pentadecane (pristane) is administered beforehand (3 to 7 days before the administration) to obtain ascites in larger amounts.

[0117] For example, an immunosuppressive agent is intraperitoneally injected in advance to the mice of the same lineage as the hybridomas to inactivate the T cells. 20 days later, 10^6 to 10^7 hybridoma clone cells are allowed to float (0.5 ml) in a serum-free medium, and this solution is intraperitoneally administered to the mice. Ascites are usually collected from the mice when abdominal distention occurs by accumulated ascites.

[0118] By this method, monoclonal antibodies are obtained with a concentration approximately 100 times higher than that in the culture solution.

[0119] The monoclonal antibodies obtained by the method can be purified by methods described in, for example, Weir, D.M.: Handbook of Experimental Immunology, Vol. I, II, III, Blackwell Scientific Publications, Oxford (1978).

[0120] Specific examples thereof include ammonium sulfate precipitation, gel filtration, ion-exchange chromatography, and affinity chromatography.

[0121] For the purification, commercially available monoclonal antibody purification kits (e.g., MAbTrap GII Kit; manufactured by Pharmacia Inc.) and the like can also be used as convenient methods.

[0122] The monoclonal antibodies thus obtained have high antigen specificity for EPHA2.

(h) Assay of monoclonal antibodies

[0123] The isotype and subclass of the monoclonal antibodies thus obtained can be determined as described below.

[0124] First, examples of identification methods include the Ouchterlony method, ELISA, and RIA.

[0125] The Ouchterlony method is convenient but requires a concentration procedure for a low concentration of monoclonal antibodies.

[0126] On the other hand, when the ELISA or RIA is used, the culture supernatant is directly reacted with an antigen-adsorbed solid phase, and further, antibodies compatible with various immunoglobulin isotypes and subclasses can be used as secondary antibodies to thereby identify the isotype or subclass of the monoclonal antibodies.

[0127] Moreover, commercially available kits for identification (e.g., Mouse Typer Kit; manufactured by Bio-Rad Laboratories, Inc.) and the like can also be used as more convenient methods.

[0128] Furthermore, the proteins can be quantified according to a Folin-Lowry method and a calculation method using absorbance at 280 nm [$1.4 \text{ (OD}_{280}) = 1 \text{ mg/ml immunoglobulin}$].

(3) Other antibodies

[0129] The antibody of the present invention encompasses the monoclonal antibody against EPHA2 as well as genetic recombinant antibodies artificially modified for the purpose of, for example, reducing xenoantigenicity against humans, for example, chimeric, humanized, and human antibodies. These antibodies can be produced according to known methods.

[0130] Examples of the chimeric antibody include an antibody having variable and constant regions derived from species different from each other and specifically include a chimeric antibody comprising mouse-derived variable regions and human-derived constant regions joined together (see Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984)).

[0131] Examples of the humanized antibody can include an antibody comprising a human-derived antibody with complementarity determining regions (CDRs) replaced with those of another species (see Nature (1986) 321, p. 522-525) and an antibody comprising a human antibody with CDR sequences and some framework amino acid residues replaced with those of another species by CDR grafting (see WO 90/07861 and US6972323).

[0132] Further examples of the antibody of the present invention can include an anti-human antibody. The anti-EPHA2 human antibody means a human antibody having only the gene sequence of a human chromosome-derived antibody. The anti-EPHA2 human antibody can be obtained by methods using human antibody-producing mice having a human chromosome fragment containing genes of human antibody H and L chains (see e.g., Tomizuka, K. et al., Nature Genetics (1997) 16, p. 133-143; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, p. 3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol. 10, p. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; and Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727).

[0133] For such transgenic animals, specifically, genetic recombinant animals in which loci of endogenous immunoglobulin heavy and light chains in non-human mammals are broken and loci of human immunoglobulin heavy and light chains are introduced

instead via yeast artificial chromosome (YAC) vectors or the like can be created by preparing knockout animals and transgenic animals and crossing these animals.

[0134] Moreover, eukaryotic cells are transformed with cDNA encoding each of such human antibody heavy and light chains, preferably vectors containing the cDNA, by gene recombination techniques, and transformed cells producing genetic recombinant human monoclonal antibodies can also be cultured to thereby obtain these antibodies from the culture supernatant.

[0135] In this context, for example, eukaryotic cells, preferably mammalian cells such as CHO cells, lymphocytes, and myelomas can be used as hosts.

[0136] Moreover, methods for obtaining phage-displayed human antibodies selected from human antibody libraries are also known (see e.g., Wormstone, I.M. et al., *Investigative Ophthalmology & Visual Science*. (2002) 43 (7), p. 2301-2308; Carmen, S. et al., *Briefings in Functional Genomics and Proteomics* (2002), 1 (2), p. 189-203; and Siriwardena, D. et al., *Ophthalmology* (2002) 109 (3), p. 427-431).

[0137] For example, a phage display method can be used, which involves causing human antibody variable regions to be expressed as a single-chain antibody (scFv) on phage surface and selecting phages binding to antigens (*Nature Biotechnology* (2005), 23, (9), p. 1105-1116).

[0138] Likewise, another phage display method can also be used, which involves causing human antibody Fab (antigen-binding fragment) to be expressed on the surface of phage and selecting phages binding to antigens (WO 97/08320 and WO 01/05950).

[0139] Genes of the phages selected based on antigen binding can be analyzed to thereby determine DNA sequences encoding human antibody variable regions binding to the antigens.

[0140] When the DNA sequence of scFv or Fab binding to the antigens is clarified, CDR sequences are extracted therefrom, and expression vectors having the sequences can be prepared and introduced into appropriate hosts, followed by gene expression to obtain human antibodies (WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438, WO 95/15388, *Annu. Rev. Immunol* (1994) 12, p. 433-455, and *Nature Biotechnology* (2005) 23 (9), p. 1105-1116).

[0141] The antibody genes can be temporarily isolated and then introduced into appropriate hosts to prepare antibodies. In such a case, appropriate hosts and expression vectors can be combined for use.

[0142] When eukaryotic cells are used as hosts, animal cells, plant cells, and eukaryotic microorganisms can be used.

[0143] Examples of the animal cells can include (1) mammalian cells, for example, monkey COS cells (Gluzman, Y. *Cell* (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L.A. *Proc. Natl. Acad. Sci. U.S.A.* (1980) 77, p. 4126-4220) of Chinese hamster ovarian cells (CHO cells, ATCC CCL-61).

[0144] Moreover, these hosts can also be modified, for use, to express antibodies having a modified sugar chain structure and an enhanced ADCC activity (antibody-dependent cytotoxic activity) or CDC activity. Examples of such hosts can include CHO cells comprising genes incorporated therein which encode antibody molecules producing antibody compositions in which sugar chains having fucose-unbound N-acetylglucosamine at the reducing ends thereof occupy 20% or more of complex-type N-glycoside linked sugar chains binding to antibody Fc regions (see WO 02/31140).

[0145] When prokaryotic cells are used, examples thereof can include *Escherichia coli* and *Bacillus subtilis*.

[0146] The antibody gene of interest is introduced into these cells by transformation, and the transformed cells are cultured in vitro to obtain antibodies.

[0147] The isotype of the antibody of the present invention is not limited, and examples thereof include IgG (IgG1, IgG2, IgG3, or IgG4), IgM, IgA (IgA1 or IgA2), IgD, and IgE and can preferably include IgG and IgM.

[0148] Moreover, the antibody of the present invention may be an antibody fragment having the antigen-binding site of the antibody or a modified form thereof.

[0149] Examples of the antibody fragment include Fab, F(ab')₂, Fv, single-chain Fv (scFv) comprising heavy and light chain Fvs

linked via an appropriate linker, a diabody, a linear antibody, and a multispecific antibody formed by antibody fragments.

[0150] Furthermore the antibody of the present invention may be a multispecific antibody having specificity for at least two different antigens.

[0151] Such a molecule usually binds two antigens (i.e., a bispecific antibody). The "multispecific antibody" according to the present invention encompasses antibodies having specificity for more (e.g., three) antigens.

[0152] The multispecific antibody used as the antibody of the present invention may be a full-length antibody or a fragment of such an antibody (e.g., a F(ab')₂ bispecific antibody). The bispecific antibody can be prepared by binding heavy and light chains (HL pairs) of two antibodies or can also be prepared by fusing hybridomas producing monoclonal antibodies different from each other to prepare bispecific antibody-producing fused cells (Millstein et al., *Nature* (1983) 305, p. 537-539).

[0153] The antibody of the present invention may be a single-chain antibody (also referred to as scFv). The single-chain antibody is obtained by linking antibody heavy and light chain V regions via a polypeptide linker (Pluckthun, *The Pharmacology of Monoclonal Antibodies*, 113 (Rosenberg and Moore ed., Springer Verlag, New York, p. 269-315 (1994)); and *Nature Biotechnology* (2005), 23, p. 1126-1136).

[0154] Methods for preparing the single-chain antibody are well known in the art (see e.g., U.S. Patent Nos. 4,946,778, 5,260,203, 5,091,513, and 5,455,030). In this scFv, the heavy and light chain V regions are linked via a linker that does not form a conjugate, preferably a polypeptide linker (Huston, J.S. et al., *Proc. Natl. Acad. Sci. U.S.A.* (1988), 85, p. 5879-5883). The heavy and light chain V regions in the scFv may be derived from the same antibodies or may be derived from different antibodies.

[0155] For example, an arbitrary single-chain peptide of 12 to 19 residues is used as the peptide linker for linking the V regions.

[0156] DNA encoding the scFv is obtained by: amplifying, as templates, the full-length sequences or partial sequences (encoding the desired amino acid sequences) of DNA encoding the heavy chain or heavy chain V region of the antibody and DNA encoding the light chain or light chain V region thereof, by a PCR method using primer pairs designed for both ends thereof; and subsequently further amplifying DNA encoding the peptide linker portion in combination with a primer pair designed to respectively link both ends of the linker sequence to the heavy and light chain sequences.

[0157] Moreover, once the DNA encoding the scFv is prepared, expression vectors containing the DNA and hosts transformed with the expression vectors can be obtained according to standard methods. Moreover, by use of the hosts, the scFv can be obtained according to standard methods.

[0158] For these antibody fragments, their genes are obtained and expressed in the same way as above, and the hosts can be allowed to produce the antibody fragments.

[0159] The antibody of the present invention may be a polyclonal antibody, which is a mixture of a plurality of anti-EPHA2 antibodies differing in amino acid sequences. One example of the polyclonal antibody can include a mixture of a plurality of antibodies differing in CDRs. A mixture of cells producing antibodies different from each other is cultured, and antibodies purified from the culture can be used as such polyclonal antibodies (see WO 2004/061104).

[0160] Antibodies obtained by binding the antibody of the present invention with various molecules such as polyethylene glycol (PEG) can also be used as the modified form of the antibody.

[0161] Furthermore, the antibody of the present invention may be a conjugate of these antibodies formed with other drugs (immunoconjugate). Examples of such an antibody can include conjugates obtained by binding these antibodies to radioactive materials or compounds having a pharmacological effect (*Nature Biotechnology* (2005) 23, p. 1137-1146).

[0162] The obtained antibodies can be purified until homogeneous. In the antibody separation and purification, any separation/purification method used for usual proteins can be used.

[0163] The antibodies can be separated and purified by appropriately selecting and combining, for example, using chromatography columns, filters, ultrafiltration, salting-out, dialysis, polyacrylamide gel electrophoresis for preparation, and isoelectric focusing (*Strategies for Protein Purification and Characterization: A Laboratory Course Manual*, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); and *Antibodies: A Laboratory Manual*. Ed Harlow and David Lane, Cold

Spring Harbor Laboratory (1988)), though the separation/purification method is not limited thereto.

[0164] Examples of chromatography include affinity chromatography, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, and adsorption chromatography.

[0165] These chromatography techniques can be performed using liquid-phase chromatography such as HPLC or FPLC.

[0166] Examples of columns used in the affinity chromatography include protein A and protein G columns.

[0167] Examples of columns based on the protein A column include Hyper D, POROS, Sepharose F.F. (Pharmacia Inc.).

[0168] Moreover, the antibodies can also be purified through their affinity for antigens using an antigen-immobilized carrier.

3. Properties of antibody of the present invention

[0169] The anti-EPHA2 antibody of some embodiments of the present invention obtained by the method has the following properties:

1. (1) one antibody of the present invention has the following properties a) to e):
 1. a) having no ability to phosphorylate EPHA2 tyrosine residues;
 2. b) having an ADCC activity against EPHA2-expressing cells;
 3. c) having a CDC activity against EPHA2-expressing cells;
 4. d) having an antitumor activity in vivo; and
 5. e) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 8):

1. 1) SH348-1,
2. 2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH348-1 (FERM BP-10836),
3. 3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in the sequence listing as complementarity determining regions in the light chain variable region,
4. 4) an antibody characterized by the following i) and ii):
 1. i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2. ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32

amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

5. 5) SH357-1,
6. 6) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH357-1 (FERM BP-10837),
7. 7) an antibody which has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing as complementarity determining regions in the light chain variable region,
8. 8) an antibody according to any one of (5) to (7), characterized by the following i) and ii):
 1. i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids sequences; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 71 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2. ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

2. (2) an antibody of another embodiment of the present invention has the following properties a) to f):
 1. a) having no ability to phosphorylate EPFA2 tyrosine residues;
 2. b) exhibiting an effect of decreasing an EPFA2 protein level;
 3. c) having an ADCC activity against EPFA2-expressing cells;
 4. d) having a CDC activity against EPFA2-expressing cells;
 5. e) having an antitumor activity in vivo; and
 6. f) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 4):

1. 1) SH348-1,
2. 2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH348-1 (FERM BP-10836),
3. 3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in the sequence listing as complementarity determining regions in the light chain variable region,
4. 4) an antibody characterized by the following i) and ii):
 1. i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



- wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and
2. ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

3. (3) an antibody of another embodiment of the present invention has the following properties a) to e):
1. a) having no ability to phosphorylate EPHA2 tyrosine residues;
 2. b) exhibiting no effect of decreasing an EPHA2 protein level;
 3. c) having an ADCC activity;
 4. d) having a CDC activity;
 5. e) having an antitumor activity in vivo; and
 6. f) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 4):

1. 1) SH357-1,
2. 2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH357-1 (FERM BP-10836),
3. 3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing as complementarity determining regions in the light chain variable region,
4. 4) an antibody having the following properties i) and ii):

1. i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 71 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2. ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents

the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

4. Pharmaceutical agent comprising anti-EPHA2 antibody

[0170] The anti-EPHA2 antibody of the present invention is useful as a pharmaceutical agent, particularly, a pharmaceutical composition intended for cancer treatment, or as an antibody for immunological diagnosis of such disease.

[0171] Preferable examples of cancer types can include, but not limited to, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme.

[0172] An embodiment of the present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of the anti-EPHA2 antibody and a pharmaceutically acceptable diluent, carrier, solubilizing agent, emulsifying agent, preservative, and/or adjuvant.

[0173] It is preferred that the substances pharmaceutically used that are acceptable in the pharmaceutical composition of the present invention should be nontoxic, at the dose or administration concentration used, to individuals that receive the pharmaceutical composition.

[0174] The pharmaceutical composition of the present invention can contain a pharmaceutical substance for changing, maintaining, or retaining pH, osmotic pressure, viscosity, transparency, color, isotonicity, sterility, stability, the rate of dissolution, the rate of sustained release, absorptivity, or permeability.

[0175] Examples of the pharmaceutical substance can include, but not limited to, the following: amino acids such as glycine, alanine, glutamine, asparagine, arginine, and lysine; antimicrobial agents; antioxidants such as ascorbic acid, sodium sulfate, and sodium bisulfite; buffers such as phosphate, citrate, and borate buffers, hydrogen carbonate, and Tris-HCl solutions; fillers such as mannitol and glycine; chelating agents such as ethylenediaminetetraacetic acid (EDTA); complexing agents such as caffeine, polyvinyl pyrrolidine, β -cyclodextrin, and hydroxypropyl- β -cyclodextrin; extenders such as glucose, mannose, and dextrin; monosaccharides, disaccharides, glucose, mannose, and other hydrocarbons such as dextrin; coloring agents; flavoring agents; diluents; emulsifying agents; hydrophilic polymers such as polyvinyl pyrrolidine; low-molecular-weight polypeptides; salt-forming counterions; antiseptics such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, and hydrogen peroxide; solvents such as glycerin, propylene glycol, and polyethylene glycol; sugar alcohols such as mannitol and sorbitol; suspending agents; surfactants such as PEG, sorbitan ester, polysorbates such as polysorbate 20 and polysorbate 80, Triton, tromethamine, lecithin, and cholesterol; stability enhancers such as sucrose and sorbitol; elasticity enhancers such as sodium chloride, potassium chloride, mannitol, and sorbitol; delivery vehicles; diluents; excipients; and/or pharmaceutical adjuvants.

[0176] The amounts of these pharmaceutical substances added are preferably 0.01 to 100 times, particularly, 0.1 to 10 times higher than the weight of the anti-EPHA2 antibody.

[0177] In this context, the present invention also encompasses a pharmaceutical composition containing an immunoliposome containing the anti-EPHA2 antibody in a liposome or the anti-EPHA2 antibody bound with a liposome (U.S. Patent No. 6214388).

[0178] The preferable composition of the pharmaceutical composition in a preparation can be determined appropriately by those skilled in the art according to applicable disease, an applicable administration route, etc.

[0179] The excipients or carriers in the pharmaceutical composition may be liquid or solid. The appropriate excipients or carriers may be injectable water, saline, cerebrospinal fluids, or other substances usually used in parenteral administration.

[0180] Neutral saline or serum albumin-containing saline can also be used as a carrier. The pharmaceutical composition can

also contain a Tris buffer (pH 7.0 to 8.5) or an acetate buffer (pH 4.0 to 5.5) as well as sorbitol or other compounds. The pharmaceutical composition of the present invention may be prepared in a freeze-dried or liquid form as an appropriate drug having the selected composition and necessary purity.

[0181] The pharmaceutical composition comprising the anti-EPHA2 antibody can also be prepared in a freeze-dried form using an appropriate excipient such as sucrose.

[0182] The pharmaceutical composition of the present invention can be prepared for parenteral administration or can also be prepared for gastrointestinal absorption.

[0183] The composition and concentration of the preparation can be determined depending on an administration method. When the anti-EPHA2 antibody contained in the pharmaceutical composition of the present invention has higher affinity for EPHA2, i.e., higher affinity (lower K_d value) for EPHA2 with respect to a dissociation constant (K_d value), the drug containing this antibody can be efficacious at a lower dose in humans. Based on this result, the dose of the pharmaceutical composition of the present invention in human can also be determined.

[0184] The dose of the anti-EPHA2 antibody in humans may be usually be approximately 0.1 to 100 mg/kg once per 1 to 180 days.

[0185] Examples of dosage forms of the pharmaceutical composition of the present invention include injections including drip, suppositories, nasal agents, sublingual agents, and transdermally absorbable agents.

[0186] The administration of the pharmaceutical composition of the present invention can inhibit the growth of EPHA2-expressing tumors.

Examples

[0187] Hereinafter, the present invention will be described more specifically with reference to the Examples. However, the present invention is not intended to be limited to them.

[0188] In the Examples below, procedures related to genetic engineering were performed according to the methods described in "Molecular Cloning", (Sambrook, J., Fritsch, E.F., and Maniatis, T., published by Cold Spring Harbor Laboratory Press, 1989) or methods described in other experimental manuals used by those skilled in the art or according to instructions included in the commercially available reagents or kits used, unless otherwise specified.

(Example 1) Preparation of plasmid

1)-1 Preparation of vectors expressing human EPHA2

1)-1-1 Preparation of a vector expressing full-length human EPHA2

[0189] cDNA encoding human EPHA2 was amplified by PCR reaction using cDNA synthesized from SK-OV-3 cell-derived total RNA as the template and a primer set:

Primer 1: 5'-ggggacaagttgtacaaaaagcaggcttcgggatcgaccgagagcgagaag-3' (sequence listing sequence ID No. 3); and

primer 2: 5'-ggggaccactttgtacaagaaagctgggtcctagatgggatcccccacagtgttcac ctggtcctt-3'
(sequence listing sequence ID No. 4).

[0190] The PCR product was incorporated into pDONR221 (manufactured by Invitrogen Corp.) using BP Clonase (manufactured by Invitrogen Corp.) to prepare an entry vector. The stop codon was removed from the EPHA2 gene in the entry vector using

GeneTailor Site-Directed Mutagenesis System (manufactured by Invitrogen Corp.) and a primer set:

Primer 3: 5'-ctgtgggagatcccatcgaccagcttcc-3' (sequence listing sequence ID No. 5); and

Primer 4: 5'-gatggggatcccccacagtgttcacctggc-3' (sequence listing sequence ID No. 6).

Recombination reaction between the obtained entry vector and the pcDNA-DEST40 Gateway Vector (manufactured by Invitrogen Corp.) was performed using LR Clonase (manufactured by Invitrogen Corp.) to prepare pcDNA-DEST40-EPHA2 (the present vector has the nucleotide sequence represented by SEQ ID NO: 7 in the sequence listing, between attB1 and attB2 sequences). Moreover, the sequence of the ORF portion of the EPHA2 gene cloned into the present vector is represented by nucleotide Nos. 33 to 2960 of SEQ ID NO: 7 in the sequence listing. Moreover, the amino acid sequence of EPHA2 is represented by SEQ ID NO: 8 in the sequence listing.

1)-1-2 Preparation of EPHA2 extracellular region expression vector

[0191] cDNA encoding a human EPHA2 extracellular region polypeptide (consisting of an amino acid sequence represented by amino acid Nos. 1 to 534 of SEQ ID NO: 8 in the sequence listing; hereinafter, abbreviated to "EPHA2-ECD") was amplified by PCR reaction using a primer set:

Primer 5: 5'-aaaaagcttatggagctccaggcagccgc-3' (sequence listing sequence ID No. 9); and

Primer 6: 5'-aaaggccctcagttgccagatccctccgg-3' (sequence listing sequence ID No. 10).

[0192] The obtained PCR product was cleaved with HindIII and Apal and cloned into the HindIII/Apal site of pcDNA3.1 (hereinafter, the resulting vector is abbreviated to "pcDNA3.1-EPHA2-ECD"; and in the description below and the drawings, the recombinant protein expressed by "pcDNA3.1-EPHA2-ECD" is referred to as "rEPHA2-ECD").

1)-1-3 Preparation of expression vectors of truncated EPHA2 proteins

[0193] To construct vectors expressing a region consisting of an amino acid sequence represented by amino acid Nos. 315 to 540 of SEQ ID NO: 8 in the sequence listing of EPHA2 (hereinafter, referred to as "FnIII-NC"), a region consisting of an amino acid sequence represented by amino acid Nos. 315 to 430 thereof (hereinafter, referred to as "FnIII-N"), or a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 540 thereof (hereinafter, referred to as "FnIII-C"), PCR reactions with pcDNA-DEST40-EPHA2 as the templates were performed using each primer set:

Primer set for FnIII-NC amplification:

Primer 7: 5'-gcaggctcatcgaaggtcgtggcgggcacctcaggaccag-3' (sequence listing sequence ID No. 11); and

Primer 8: 5'-gtacaagaaagctgggtgctagccccaatcaccgccaag-3' (sequence listing sequence ID No. 12);

Primer set for FnIII-N amplification: Primer 7, and

Primer 9: 5'-gtacaagaaagctgggtgctaggcagctacggaagctgcgg-3' (sequence listing sequence ID No. 13);

Primer set for FnIII-C amplification:

Primer 10: 5'-gcaggctcatcgaaggtcgtgggagctccgtactgccagt-3' (sequence listing sequence ID No. 14); and

Primer 8.

[0194] To add attB1 and attB2 sites to both ends of the obtained PCR products, PCR reaction with each PCR product as a template was performed using a primer set:

Primer 11: 5'-ggggacaagttgtacaaaaagcaggctcatcgaaggtcgtggg-3' (sequence listing sequence ID No. 15); and

Primer 12: 5'-ggggaccactttgtacaagaaagctgggt-3' (sequence listing sequence ID No. 16).

[0195] The PCR products obtained in this procedure were incorporated into pDONR221 using BP Clonase to prepare entry vectors. Recombination reactions between each entry vector and a destination vector prepared by cleaving the NdeI and BamHI sites of pET15b (manufactured by Novagen) with restriction enzymes, blunting the cleaved sites, and then ligating Reading Frame Cassette C.1 of Gateway Vector Conversion System (manufactured by Invitrogen Corp.) into the blunted sites were performed using LR Clonase in order to prepare expression vectors (hereinafter, recombinant proteins expressed by the FnlII-NC-, FnlII-N-, and FnlII-C-incorporated expression vectors are referred to as rFnlII-NC, rFnlII-N, and rFnlII-C, respectively).

1)-2 Preparation of human EPHB2 extracellular region expression vector

[0196] cDNA encoding human EPHB2 was obtained by PCR reaction using cDNA synthesized from HCC70 cell-derived total RNA as the template and a primer set:

Primer 13: 5'-ggggacaagtgtgtacaaaaagcaggcttcgccccgggaagcgcagcc-3' (sequence listing sequence ID No. 17); and

Primer 14: 5'-ggggaccactttgtacaagaaagctgggtcctaaacctccacagactgaatctggtt catctg-3' (sequence listing sequence ID No. 18).

[0197] The nucleotide sequence of human EPHB2 cDNA is represented by SEQ ID NO: 19 in the sequence listing.

[0198] The amino acid sequence thereof is represented by SEQ ID NO: 20 in the sequence listing. PCR reaction was performed using a primer set for amplifying cDNA encoding a human EPHB2 extracellular region (region consisting of an amino acid sequence represented by amino acid Nos. 1 to 542 of SEQ ID NO: 20 in the sequence listing) (hereinafter, abbreviated to "EPHB2-ECD"):

Primer 15: 5'-aaaaagcttatggctctgcggaggctgggg-3' (sequence listing sequence ID No. 21); and

Primer 16: 5'-aaagatatctcatggcaacttctcctggat-3' (sequence listing sequence ID No. 22).

[0199] The obtained PCR product was cleaved with HindIII and EcoRV and cloned into the HindIII/EcoRV site of pcDNA3.1 (hereinafter, the resulting vector is abbreviated to "pcDNA3.1-EPHB2-ECD"; and in the description below and the drawings, the recombinant protein expressed by "pcDNA3.1-EPHB2-ECD" is referred to as rEPHB2-ECD).

1)-3 Preparation of human ERBB2 expression vector

[0200] PCR reaction with Human clone collection (manufactured by STRATAGENE, #C33830) as the template was performed using a primer set:

Primer 17: 5'-caccatggagctggcggccttg-3' (sequence listing sequence ID No. 23); and

Primer 18: 5'-tcccactggcagctccagacc-3' (sequence listing sequence ID No. 24).

[0201] The obtained PCR product was incorporated into pENTR/D-TOPO (manufactured by Invitrogen Corp.) using pENTR Directional TOPO Cloning kit (manufactured by Invitrogen Corp.) to prepare an entry vector. To repair mutations caused by amino acid substitution, the entry vector was digested with EcoRI, and a fragment containing the pENTR/D-TOPO-derived sequence among the obtained fragments was ligated with the second largest fragment (approximately 1.6 kbp) among fragments obtained by digested Human clone collection (manufactured by STRATAGENE, #C14640) with EcoRI. Recombination reactions between the obtained entry vector and the pcDNA-DEST40 Gateway vector were performed using LR Clonase in order to prepare pcDNA-DEST40-ERBB2 (the vector has the nucleotide sequence represented by SEQ ID NO: 25 in the sequence listing, between attB1 and attB2 sequences).

(Example 2) Preparation of monoclonal antibody

2)-1 Preparation of antigen

[0202] To express EPHA2-ECD, FreeStyle 293-F cells (manufactured by Invitrogen Corp.) were transfected with pcDNA3.1-EPHA2-ECD using 293fectin (manufactured by Invitrogen Corp.) and cultured at 37°C in 8% CO₂ for 5 days. After the culture, the culture supernatant was collected by centrifugation and used as a source for rEPA2-ECD purification. The obtained culture supernatant was dialyzed against 20 mM Tris-HCl, pH 7.5, using a dialysis tube having a molecular cutoff of 15000, then filtered through a filter (0.45 µm, PES), and then applied to HiPrep 16/10 Q XL (manufactured by GE Healthcare Bio-Sciences Corp.) equilibrated with 20 mM Tris-HCl, pH 7.5. Elution was performed with a linear concentration gradient of NaCl (20 mM Tris-HCl, pH 7.5, 0-1 M NaCl). An aliquot of the eluted fractions was separated by SDS-polyacrylamide gel electrophoresis (hereinafter, abbreviated to "SDS-PAGE"). Then, the gel was stained with Coomassie Brilliant Blue (hereinafter, abbreviated to "CBB-stained") to confirm rEPA2-ECD-containing fractions. Next, the rEPA2-ECD-containing fractions were combined and applied to HiLoad 26/60 Superdex 200 pg (manufactured by GE Healthcare Bio-Sciences Corp.) equilibrated with PBS. After elution with PBS, an aliquot of the eluted fractions was separated by SDS-PAGE. Then, the gel was CBB-stained to confirm rEPA2-ECD-containing fractions. The rEPA2-ECD-containing fractions were combined and used as an antigen for immunization and an antigen for epitope determination. The protein concentration was measured using BCA Protein Assay Reagent (manufactured by PIERCE).

2)-2 Immunization

[0203] 4- to 6-week-old BALB/cAnNCrCrIj mice (Charles River Laboratories Japan, Inc.) were used. On day 0, a mixture of 50 µg of rEPA2-ECD and Adjuvant Complete Freund H37 Rv (manufactured by Wako Pure Chemical Industries, Ltd.) (1:1 in terms of volume ratio) was subcutaneously administered to the mouse dorsal region. Likewise, a mixture of 50 µg of rEPA2-ECD and TiterMax Gold Adjuvant (manufactured by Sigma-Aldrich, Inc.) (1:1 in terms of volume ratio) was subcutaneously administered to the dorsal region of another individual. On days 22 and 36, a mixture of 50 µg of rEPA2-ECD and Adjuvant Incomplete Freund (manufactured by Wako Pure Chemical Industries, Ltd.) (1:1 in terms of volume ratio) was subcutaneously administered to the dorsal region of each mouse. On day 53, 50 µg of rEPA2-ECD was intraperitoneally administered to each mouse. On day 56, the mouse spleen was collected and used in hybridoma preparation.

2)-3 Hybridoma preparation

[0204] Cell fusion between the spleen cells and mouse myeloma P3X63Ag8U.1 cells was performed using PEG4000 (manufactured IBL (Immuno-Biological Laboratories, Co., Ltd.)) to prepare hybridomas. A culture supernatant of the obtained hybridomas was used for screening anti-EPHA2 antibody-producing hybridomas.

2)-4 Antibody screening

2)-4-1 Preparation of cells expressing a gene encoding an antigen

[0205] 293T cells were seeded at 5×10^4 cells/cm² onto a collagen type I-coated flask (manufactured by IWAKI) and cultured overnight at 37°C in 5% CO₂ in DMEM containing 10% FBS. On the next day, the 293T cells were transfected with pcDNA-DEST40-EPHA2 or pcDNA-DEST40-ERBB2 as a control using Lipofectamine 2000 (manufactured by Invitrogen Corp.) and further incubated overnight at 37°C in 5% CO₂. On the next day, the transfected 293T cells were treated with trypsin, then washed with DMEM containing 10% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in Cell-ELISA and flow cytometry analysis.

2)-4-2 Cell-ELISA

[0206] The cell suspension prepared in the paragraph 2)-4-1 was centrifuged, and the supernatant was removed. Then, the EPHA2-expressing 293T cells and the ERBB2-expressing 293T cells were separately suspended by the addition of the hybridoma culture supernatant and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, the cells were suspended by the addition of Goat anti-Mouse IgG, Peroxidase Conjugated (manufactured by Millipore (Chemicon), #AP181P) diluted 500 times with PBS containing 5% FBS, and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, OPD Color Developing Solution (o-phenylenediamine dihydrochloride (manufactured by Wako Pure Chemical Industries, Ltd.) and H₂O₂ were dissolved at concentrations of 0.4 mg/ml and 0.6% (v/v), respectively, in an OPD solution (0.05 M trisodium citrate, 0.1 M disodium hydrogen phosphate dodecahydrate, pH 4.5)) was added at 100 µl/well. Color reaction was performed with stirring and terminated by the addition of 1 M HCl at 100 µl/well. The cells were precipitated by centrifugation, and the supernatant was then transferred to a new 96-well flat-bottom microplate. The absorbance at 490 nm was measured using a plate reader (ARVO, PerkinElmer). To select hybridomas producing antibodies which specifically bind to EPHA2 expressed on the surface of the cell membrane, hybridomas producing a culture supernatant exhibiting higher absorbance in the EPHA2-expressing 293T cells than in the ERBB2-expressing 293T cells (controls) were selected to be positive for production of anti-EPHA2 antibody.

2)-4-3 Flow cytometric analysis

[0207] To eliminate false positives in Cell-ELISA, antibodies produced by the hybridoma determined to be positive in Cell-ELISA were further examined for their binding specificities for EPHA2 by flow cytometry. The cell suspension prepared in paragraph 2)-4-1 was centrifuged, and the supernatant was removed. Then, the EPHA2-expressing 293T cells and the ERBB2-expressing 293T cells were separately suspended by the addition of the hybridoma culture supernatant and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, the cells were suspended by the addition of "Fluorescein-conjugated goat IgG fraction to mouse IgG" (Whole Molecule) (manufactured by ICN Pharmaceuticals, Inc., #55493) diluted 1000 times with PBS containing 5% FBS, and incubated at 4°C for 1 hour. The cells were washed twice with PBS containing 5% FBS and then resuspended in PBS containing 5% FBS and further containing 2 µg/ml 7-aminoactinomycin D (manufactured by Invitrogen Corp. (Molecular Probes)), followed by analysis using a flow cytometer (FC500, Beckman Coulter, Inc.). The data was analyzed using Flowjo (Tree Star, Inc.). 7-aminoactinomycin D-positive dead cells were excluded using a gate. Then, the FITC fluorescence intensity histograms of live cells were plotted. Hybridomas producing samples that provided stronger fluorescence intensity in the fluorescence intensity histogram of the EPHA2-expressing 293T cells than in the histogram of the ERBB2-expressing 293T cells as controls were obtained as anti-EPHA2 antibody-producing hybridomas.

2)-5 Separation of hybridoma into single clones

[0208] The anti-EPHA2 antibody-producing hybridomas were diluted with ClonaCell-HY Selection Medium D (manufactured by StemCell Technologies, #03804) and cultured, and the formed colonies were collected as single clones. The collected clones were separately cultured and examined for their binding activities for EPHA2 using the culture supernatants in the same way as in paragraph 2)-4-3 to establish hybridomas producing anti-EPHA2 monoclonal antibodies (SH348-1, SH357-1, Ab57-1, Ab65-1, Ab96-1, Ab100-1, Ab105-1, Ab106-13, Ab136-1, Ab148-1, Ab151-4, Ab230-1, Ab373-1, and Ab382-1).

2)-6 Confirmation of binding activity of monoclonal antibody for cancer cell line

[0209] Whether or not the monoclonal antibodies obtained in paragraph 2)-5 bound to cancer cells highly expressing EPHA2 was studied by the flow cytometric method in the same way as in paragraph 2)-4-3. A human breast cancer cell line (MDA-MB-231), a human lung cancer cell line (A549), and a human prostate cancer cell line (PC-3) were used instead of the transfected 293T cells. As a result, all the established monoclonal antibodies were confirmed to bind to these cancer cell lines.

2)-7 Isotype determination of monoclonal antibody

[0210] The isotypes of the monoclonal antibodies were determined using Mouse monoclonal isotyping kit (manufactured by AbD Serotec). As a result, the isotypes were IgG1 (Ab57-1 and Ab230-1), IgG2a (SH348-1, SH357-1, Ab65-1, Ab96-1, Ab100-1, Ab136-1, Ab148-1, and Ab151-4), and IgG2b (Ab105-1, Ab106-13, Ab373-1, and Ab382-1).

2)-8 Preparation of monoclonal antibody

[0211] The monoclonal antibodies were purified from ascites of hybridoma-transplanted mice or a hybridoma culture supernatant (hereinafter, referred to as a "source for antibody purification").

[0212] The mouse ascites were prepared as follows: first, 7- to 8-week-old BALB/cAJcl-nu/nu mice (CLEA Japan, Inc.) were treated with pristane (manufactured by Sigma-Aldrich, Inc.). Approximately 3 weeks later, the hybridomas washed with saline were intraperitoneally transplanted in an amount of 1×10^7 cells/mouse. 1 to 2 weeks later, ascites accumulated in the peritoneal cavity was collected, then sterilized through a 0.22- μ m filter, and used as a source for antibody purification.

[0213] The hybridoma culture supernatant was prepared using CELLline (manufactured by BD Biosciences). The hybridomas were cultured according to the manufacturer's instructions except that ClonaCell-HY Growth Medium E (manufactured by StemCell Technologies, #03805) was used as a medium. The collected culture supernatant was filtered through a 0.45- μ m filter and used as a source for antibody purification.

[0214] The antibodies were purified using an affinity column comprising Recombinant Protein A rPA50 (manufactured by Repligen Corp.) immobilized on Formyl-Cellulofine (manufactured by Seikagaku Corp.) (hereinafter, abbreviated to "Formyl-Cellulofine Protein A") or HiTrap MabSelect SuRe (manufactured by GE Healthcare Bio-Sciences Corp.). For the Formyl-Cellulofine Protein A, the source for antibody purification was diluted three times with Binding Buffer (3 M NaCl, 1.5 M glycine, pH 8.9) and applied to the column, which was then washed with Binding Buffer, followed by elution with 0.1 M citric acid, pH 4.0. On the other hand, for the HiTrap MabSelect SuRe, the source for antibody purification was added to the column, which was then washed with PBS, followed by elution with 2 M arginine-HCl, pH 4.0. The antibody eluate was neutralized, and the buffer was then replaced with PBS.

[0215] The antibody concentrations were determined by eluting the antibodies bound with POROS G 20 μ m Column, PEEK, 4.6 mm $\times$ 100 mm, 1.7 ml (Applied Biosystems) and measuring the absorbance (O.D. 280 nm) of the eluate. Specifically, the antibody sample diluted with PBS was applied to POROS G 20 μ m equilibrated with Equilibrating Buffer (30.6 mM sodium dihydrogen phosphate dodecahydrate, 19.5 mM monopotassium phosphate, 0.15 M NaCl, pH 7.0). The column was washed with Equilibrating Buffer, and the antibody bound to the column was then eluted with an eluent (0.1% (v/v) HCl, 0.15 M NaCl). The peak area of the absorbance (O.D. 280 nm) of the eluate was measured, and the concentration was calculated according to the following equation:

$$\text{Concentration of antibody sample (mg/ml)} = \frac{(\text{Peak area of antibody sample})}{(\text{Peak area of standard (human IgG1)})} \times \text{Concentration of standard (mg/ml)} \times \text{Dilution factor}.$$

[0216] Moreover, the concentration of endotoxin contained in the obtained antibodies was measured using Endospecy ES-50M Set (Seikagaku Corp., #020150) and Endotoxin Standard CSE-L Set (Seikagaku Corp., #020055) and was confirmed to be 1 EU/mg or lower. The resulting antibodies were used in the subsequent experiments.

(Example 3) Properties of SH348-1 and SH357-1

[0217] 3)-1 Study of anti-EPHA2 antibody for its activity of inducing phosphorylation of EPHA2 tyrosine residues and its activity of inducing decrease in EPHA2 protein level

[0218] 3)-1-1 Preparation of antibody-stimulated cell lysate MDA-MB-231 cells suspended in RPMI1640 containing 10% FBS, 50 units/ml penicillin, and 50 μ g/ml streptomycin (hereinafter, abbreviated to "10% FBS-containing RPMI1640 (with antibiotics)") were seeded at 6×10^5 cells/well onto a 6-well dish and cultured overnight at 37°C in 5% CO₂. On the next day, the medium was discarded, and RPMI1640 was added thereto. The cells were further cultured overnight at 37°C in 5% CO₂. On the next day, SH348-1, SH357-1, Mouse IgG_{2A} Isotype Control (in the description below and the drawings, abbreviated to "mIgG_{2A}"; manufactured by R&D Systems, Inc., #MAB003) as an isotype control antibody, Recombinant Mouse Ephrin-A1/Fc Chimera (in the description below and the drawings, abbreviated to "Ephrin-A1/Fc"; manufactured by R&D Systems, Inc., #602-A1-200) as a soluble EPHA2 ligand, and Recombinant Human IgG₁ Fc (in the description below and the drawings, abbreviated to "hG₁Fc";

manufactured by R&D Systems, Inc., #110-HG-100) as a control protein for the soluble ligand were separately diluted at a concentration shown in Figure 1 or 2 (SH348-1, SH357-1, and mlgG2a: 10 µg/ml or 50 µg/ml in Figure 1A and 50 µg/ml in Figure 2A, Ephrin-A1/Fc and hG₁Fc: 1 µg/ml in Figures 1 and 2) with RPMI1640. The resulting solution was added to the MDA-MB-231 cells after discarding of the medium and incubated at 37°C in 5% CO₂ for the predetermined time in 5% CO₂. Moreover, in the experiments in the presence of a cross-linking antibody, SH348-1, SH357-1, or mlgG2a and Goat anti-mouse IgG, Fcγ fragment specific (min X Hu, Bov, Hrs Sr Prot) (manufactured by Jackson ImmunoResearch Laboratories, Inc., #115-005-071) as a cross-linking antibody were mixed at each concentration of 10 µg/ml or 50 µg/ml (Figure 1B) and 50 µg/ml (Figure 2B) in RPMI1640. The resulting solution was added to the MDA-MB-231 cells after discarding of the medium and incubated at 37°C in 5% CO₂ for the predetermined time. At the predetermined time, the supernatant was removed, and the cells were lysed by the addition of 1×Cell Lysis Buffer (manufactured by Cell Signaling Technology, Inc.) containing 1 mM PMSF (manufactured by Sigma-Aldrich, Inc.) and centrifuged at 15000 rpm for 5 minutes. The supernatants of cell lysates were used as samples in immunoprecipitation and Western blotting. The protein concentrations of the samples were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

3)-1-2 Verification of activity of inducing phosphorylation of EPHA2 tyrosine residues

[0219] To immunoprecipitate EPHA2, first, 8 µg of anti-Eck/EphA2, clone D7 (in the description below and the drawings, abbreviated to "anti-EPHA2 antibody (D7)"; manufactured by Millipore (Upstate), #05-480) was added to 25 µl of a suspension of Protein G magnetic beads (manufactured by NEW ENGLAND BioLabs, Inc.) per sample, and the mixture was inverted for mixing at 4°C for 2 hours.

[0220] Then, FBS was added thereto at a final concentration of 10%, and the mixture was further inverted for mixing at 4°C for 30 minutes. The beads were washed three times with 1×Cell Lysis Buffer containing 1 mM PMSF. Then, 200 µg of the cell lysate supernatants prepared in paragraph 3)-1-1 were added thereto, and the mixtures were inverted for mixing overnight at 4°C. On the next day, the beads were washed three times with 1×Cell Lysis Buffer containing 1 mM PMSF. Then, SDS-Sample Buffer (56.3 mM Tris-HCl, pH 6.8, 1.8% (w/v) SDS, 9% glycerol, 0.72 M 2-mercaptoethanol, 0.045 mg/ml bromophenol blue) was added to the beads, and the mixtures were heated at 98°C for 5 minutes. The proteins dissociated from the beads were separated by SDS-PAGE.

[0221] To perform Western blotting, the proteins were transferred from the gels to polyvinylidene difluoride membranes (hereinafter, abbreviated to a "PVDF membrane"; 0.45 µm in pore size; manufactured by Millipore). After the transfer, the PVDF membranes were blocked by shaking in Blocking Solution (one pouch of Block Ace powder (manufactured by Dainippon Sumitomo Pharma Co., Ltd. (Snow Brand Milk Products Co., Ltd.)) was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). First, to detect the immunoprecipitated EPHA2, the PVDF membranes thus blocked were soaked in an anti-EPHA2 antibody (D7) solution diluted to 0.25 µg/ml with Blocking Solution, and shaken at room temperature for 1 hour. The PVDF membranes were washed for 10 minutes three times with TBST (50 mM Tris-HCl, pH 8.0, 138 mM NaCl, 2.7 mM KCl, 0.1% (v/v) Tween 20). Then, the PVDF membranes were soaked in an Anti-Mouse Ig, HRP-Linked Whole Ab Sheep (manufactured by GE Healthcare Bio-Sciences Corp.) solution diluted 3000 times with TBST, and shaken at room temperature for 30 minutes. The PVDF membranes were further washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus (manufactured by GE Healthcare Bio-Sciences Corp.).

[0222] Next, to remove the antibodies from these PVDF membranes, the PVDF membranes were soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol) and shaken at 55°C for 30 minutes. Then, the PVDF membranes were soaked in Quenching Solution (TBST containing 1% (v/v) H₂O₂ and 0.1% (w/v) NaN₃), then shaken at room temperature for 20 minutes, and further washed for 10 minutes three times with TBST. To detect the phosphorylated state of the EPHA2 tyrosine residues, these PVDF membranes were blocked by shaking in sodium azide-free Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 was then added at a final concentration of 0.1% (v/v)). Then, the PVDF membranes were soaked in an Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (in the figures, abbreviated to "4G10 antibody"; manufactured by Millipore (Upstate), #16-184) solution diluted 10000 times with sodium azide-free Blocking Solution, and shaken at room temperature for 1 hour. The PVDF membranes were washed for 10 minutes three times with TBST and then further washed for 5 minutes three times with H₂O. Signals were detected on a film for chemiluminescence using ECL Plus.

[0223] As a result, by the addition of the soluble ligand Ephrin-A1/Fc, EPHA2 tyrosine residues were phosphorylated in 10 minutes. By contrast, when the antibodies SH348-1 and SH357-1 were added at a concentration of 10 µg/ml or 50 µg/ml, the

effect of inducing the phosphorylation of EPHA2 tyrosine residues as seen by the ligand was not observed at all in the predetermined times (10 minutes, 30 minutes, and 60 minutes) (Figure 1A). Likewise, even in the presence of the cross-linking antibody, the effect of inducing the phosphorylation of EPHA2 tyrosine residues as seen in the ligand was not observed in the presence of antibodies SH348-1 and SH357-1 (Figure 1B).

3)-1-3 Verification of activity of inducing decrease in EPHA2 protein level

[0224] 10 µg of the cell lysate supernatants prepared in paragraph 3)-1-1 were separated by SDS-PAGE. Then, the proteins in the gel were transferred to PVDF membranes and subjected to Western blotting using an anti-EPHA2 antibody (D7) and Monoclonal Anti-β-Actin clone AC-15 (in the description below and the figures, abbreviated to "anti-β-actin antibody"; manufactured by Sigma-Aldrich, Inc., #A-5441) as a control for the sample protein level. Specifically, after the transfer, the PVDF membranes were blocked by shaking in Blocking Solution and then cut with a razor into two pieces centered around a molecular weight of 70 kDa. The PVDF membranes containing 70 kDa or larger proteins were soaked in an anti-EPHA2 antibody (D7) solution diluted to 0.25 µg/ml with Blocking Solution, while the PVDF membranes containing 70 kDa or smaller proteins were soaked in an anti-β-actin antibody solution diluted 1000 times with Blocking Solution. Each PVDF membrane was shaken at room temperature for 1 hour. Each PVDF membrane was washed for 10 minutes three times with TBST. Then, each PVDF membrane was soaked in an Anti-Mouse Ig, HRP-Linked Whole Ab Sheep solution diluted 3000 times with TBST, and shaken at room temperature for 30 minutes. Each PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected using ECL Plus and NightOWL LB983 (Berthold Technologies GmbH & Co. KG). The signal intensity of the bands was quantified using Gel-Pro Analyzer Version 4.5 for Windows (registered trademark; Media Cybernetics, Inc.).

[0225] As a result, by the addition of the soluble ligand Ephrin-A1/Fc, a significant decrease in EPHA2 protein level was observed (Figures 2A and 2B). By the addition of the antibody SH348-1, a decrease in EPHA2 protein level, albeit weaker than the activity of the ligand, was observed both in the presence and absence of the cross-linking antibody (Figures 2A and 2B). On the other hand, in the antibody SH357-1, almost no change in EPHA2 protein level was observed regardless of the presence or absence of the cross-linking antibody (Figures 2A and 2B).

[0226] To analyze the EPHA2 protein level after 24 hours of the SH348-1 addition, an average of {the signal intensity of the EPHA2 band/the signal intensity of the β-actin band} was calculated from three experimental results corrected with a ligand/antibody-nonsupplemented sample. As a result, the value after 24 hours of the SH348-1 addition was 70% in the absence of the cross-linking antibody and 69% in the presence of the cross-linking antibody, when the value after 24 hours of the mlgG2a addition is defined as 100%.

3)-2 ADCC activity

3)-2-1 Preparation of effector cells

[0227] The spleen was aseptically collected from CAnN.Cg-Foxn1^{nu}/CrJ nude mice (Charles River Laboratories Japan, Inc.). The collected spleen was homogenized with two slide glasses and hemolyzed using BD Pharm Lyse (manufactured by BD Biosciences, #555899). The obtained spleen cells were suspended in phenol red-free RPMI1640 (manufactured by Invitrogen Corp.) containing 10% Fetal Bovine Serum, Ultra-low IgG (manufactured by Invitrogen Corp.) (hereinafter, abbreviated to a "medium for ADCC") and passed through a cell strainer (40 µm in pore size; manufactured by BD Biosciences). Then, the number of live cells was counted by a trypan blue exclusion test. The spleen cell suspension was centrifuged, and the medium was then removed. The cells were resuspended at a live cell density of 1.5×10^7 cells/ml in a medium for ADCC and used as effector cells.

3)-2-2 Preparation of target cells

[0228] MDA-MB-231, A549, or PC-3 cells were treated with trypsin. The cells were washed with RPMI1640 containing 10% FBS and then resuspended in RPMI1640 containing 10% FBS. Each cell (4×10^6 cells) was mixed with Chromium-51 (5550 kBq) sterilized through a 0.22-µm filter, and labeled therewith at 37°C in 5% CO₂ for 1 hour. The labeled cells were washed three times with a medium for ADCC and resuspended at a concentration of 2×10^5 cells/ml in a medium for ADCC to prepare target cells.

3)-2-3 ⁵¹Cr release assay

[0229] The target cells (2×10^5 cells/ml) were dispensed at 50 μ l/well to a 96-well U-bottom microplate. 50 μ l of SH348-1, SH357-1, or an isotype control antibody (mIgG2a) diluted to 2.5 μ g/ml (in terms of a final concentration after addition of the effector cells) with a medium for ADCC was added thereto and incubated at 4°C for 1 hour. 100 μ l of the effector cells (1.5×10^7 cells/ml) was added thereto and incubated overnight at 37°C in 5% CO₂. On the next day, the supernatant was collected into LumaPlate (manufactured by PerkinElmer). The dose of released gamma rays was measured using a gamma counter. The cell lysis rate attributed to the ADCC activity was calculated according to the following equation:

$$\text{Cell lysis rate (\%)} = (A-B)/(C-B) \times 100$$

1. A: counts in sample wells
2. B: an average (n = 3) of counts in spontaneous release (antibody/effector cell-nonsupplemented wells). Instead of the antibody and the effector cells, 50 μ l and 100 μ l, respectively, of a medium for ADCC were added. The other procedures were performed in the same way as in the sample wells.
3. C: an average (n = 3) of counts in the maximum release (wells containing the target cells dissolved in a detergent). 50 μ l of a medium for ADCC was added instead of the antibody, and 100 μ l of a medium for ADCC containing 2% (v/v) Triton-X100 was added instead of the effector cells. The other procedures were performed in the same way as in the sample wells.

[0230] Figure 3 shows an average of three experiments, wherein the error bar represents a standard deviation, and the P value was calculated by Student's t-test. As a result, the antibody SH348-1 exhibited cell lysis activities of 8.2%, 9.1%, and 4.7% against the MDA-MB-231 cells (Figure 3A), the A549 cells (Figure 3B), and the PC-3 cells (Figure 3C), respectively. The antibody SH357-1 exhibited cell lysis activities of 8.8%, 13.0%, and 9.0% against the MDA-MB-231 cells (Figure 3A), the A549 cells (Figure 3B), and the PC-3 cells (Figure 3C), respectively. These results demonstrated that both the antibodies have an ADCC activity against MDA-MB-231 cells, A549 cells, and PC-3 cells.

3)-3 CDC activity

[0231] MDA-MB-231, A549, or PC-3 cells suspended in 10% FBS-containing RPMI1640 (with antibiotics) were seeded at 5000 cells/well onto a 96-well microplate and cultured overnight at 37°C in 5% CO₂. On the next day, SH348-1, SH357-1, or an isotype control antibody (mIgG2a) diluted to 25 μ g/ml (in terms of a final concentration after addition of complements) with 10% FBS-containing RPMI1640 (with antibiotics) was added thereto and incubated at 4°C for 1 hour. Rabbit complements (manufactured by CEDARLANE, #CL3051) diluted to 30% with RPMI1640 were added thereto at a final concentration of 5%, then incubated at 37°C in 5% CO₂ for 1 hour, and further left standing at room temperature for 30 minutes. To measure the cell viability, CellTiter-Glo Luminescent Cell Viability Assay (manufactured by Promega Corp.) was added in an amount equal to that of the culture solution, and the mixture was stirred at room temperature for 10 minutes. Then, the amount of light emitted was measured using a plate reader. The cell viability was calculated according to the following equation:

$$\text{Cell viability (\%)} = (a-b)/(c-b) \times 100$$

1. a: the amount of light emitted from sample wells
2. b: an average (n = 8) of the amount of light emitted as a background (cell/antibody-nonsupplemented wells). 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the seeded cells, in an amount equal to that of the cell suspension, and 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the antibody, in an amount equal to that of the antibody dilution. The other procedures were performed in the same way as in the sample wells.
3. c: an average (n = 3) of the amount of light emitted from antibody-nonsupplemented wells. 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the antibody, in an amount equal to that of the antibody dilution. The other procedures were performed in the same way as in the sample wells.

[0232] Figure 4 shows an average of three experiments, wherein the error bar represents a standard deviation, and the P value was calculated by Student's t-test. As a result, the antibody SH348-1 induced 44%, 31%, and 41% decreases in the cell viability of the MDA-MB-231 cells (Figure 4A), the A549 cells (Figure 4B), and the PC-3 cells (Figure 4C), respectively, in the presence of the complements. The antibody SH357-1 also induced 65%, 60%, and 65% decreases in the cell survival rates of the MDA-MB-

231 cells (Figure 4D), the A549 cells (Figure 4E), and the PC-3 cells (Figure 4F), respectively, in the presence of the complements. These results demonstrated that both the antibodies have a CDC activity against MDA-MB-231 cells, A549 cells, and PC-3 cells.

3)-4 Epitope determination

3)-4-1 Preparation of truncated EPHA2 polypeptides (rFnIII-NC, rFnIII-N, and rFnIII-C)

[0233] *Escherichia coli* BL21 and *Escherichia coli* Origami (DE3) (manufactured by Novagen) were separately transformed with the expression plasmid prepared in paragraph 1)-1-3, and cultured in an LB medium supplemented with 50 µg/ml ampicillin (manufactured by Sigma-Aldrich, Inc.). Expression of truncated EPHA2 polypeptides was induced using Autoinduction System (manufactured by Novagen) for BL21 and the addition of 0.5 mM IPTG for Origami (DE3). The bacterial cells were collected by centrifugation at 6000 rpm for 20 minutes, then suspended in a homogenizing buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 0.1% (v/v) Triton-X100, 10% (v/v) glycerol), and then sonicated on ice. The supernatant was collected by centrifugation at 14000 rpm for 15 minutes and applied to 0.5 ml of Ni-NTA (manufactured by Invitrogen Corp.). The Ni-NTA was washed with a washing buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 50 mM imidazole, 10% (v/v) glycerol), followed by elution with an eluting buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 400 mM imidazole, 10% (v/v) glycerol). The eluted samples were further purified by gel filtration column chromatography (Superdex 75 10/300; manufactured by GE Healthcare Bio-Sciences Corp.) using PBS as a solvent. The protein concentrations of the obtained recombinant proteins were measured using Protein Assay (manufactured by Bio-Rad Laboratories, Inc).

3)-4-2 Preparation of EPHB2 extracellular region polypeptide (rEPHB2-ECD)

[0234] To express EPHB2-ECD, FreeStyle 293-F cells were transfected with pcDNA3.1-EphA2-ECD using 293fectin and cultured at 37°C in 8% CO₂ for 72 hours. After the culture, the culture solution was collected by centrifugation and used as a source for rEPHB2-ECD purification. The obtained culture supernatant was dialyzed against 20 mM Tris-HCl, pH 7.5, using a dialysis tube having a molecular cutoff of 15000, then filtered through a filter (0.45 µm, PES), and then applied to HiPrep 16/10 Q XL equilibrated with 20 mM Tris-HCl, pH 7.5. Elution was performed with a linear concentration gradient of NaCl (20 mM Tris-HCl, pH 7.5, 0-1 M NaCl). An aliquot of the eluted fractions was separated by SDS-PAGE. Then, the gel was CBB-stained to confirm that there were rEPHB2-ECD-containing fractions. Next, the rEPHB2-ECD-containing fractions were combined and applied to HiLoad 26/60 Superdex 200 pg equilibrated with PBS. After elution with PBS, an aliquot of the elution fractions was separated by SDS-PAGE. Then, the gel was CBB-stained to confirm that there were rEPHB2-ECD-containing fractions. The rEPHB2-ECD-containing fractions were combined and used as an antigen for epitope determination. The protein concentration was measured using BCA Protein Assay Reagent.

3)-4-3 Determination of binding sites in antigens by ELISA

[0235] rEPA2-ECD, rFnIII-NC, rFnIII-N, rFnIII-C, or a control protein rEPHB2-ECD was diluted to 1 µg/ml with PBS, then dispensed at 100 µl/well onto an immunoplate (manufactured by Nunc, #442404), and incubated overnight at 4°C to thereby adsorb the protein to the plate. On the next day, the solution in the wells was removed, and a Block Ace solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS was dispensed at 200 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and SH348-1, SH357-1, or an isotype control antibody (mIgG2a) diluted to 5 µg/ml with Diluting Buffer (PBS, 0.05% (v/v) Tween 20) was then added at 50 µl/well. The plate was incubated at room temperature for 1 hour. Then, the solution in the wells was removed, and the wells were washed twice with a diluting buffer. Goat anti-Mouse IgG, Peroxidase Conjugated, diluted 3000 times with Diluting Buffer was added at 50 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was performed with stirring by the addition of OPD Color Developing Solution at 100 µl/well. After color development, the color reaction was terminated by the addition of 1 M HCl at 100 µl/well. The absorbance at 490 nm was measured using a plate reader.

[0236] Figure 5A shows the EPHA2 domain structure prediction (NCBI CDD version 2.11, CBS TMHMM Server v.2.0) and the positions of EPHA2-ECD, FnIII-NC, FnIII-N, and FnIII-C in EPHA2. Ligand-BD represents a ligand-binding domain, FN3 represents

a fibronectin type 3 domain, TM represents a transmembrane region, Trk kinase represents a tyrosine kinase domain, and SAM represents a SAM domain.

[0237] Recombinant proteins of the EPHA2 extracellular region (EPHA2-ECD), the region containing two fibronectin type 3 domains (FnIII-NC), the region containing the N-terminal fibronectin type 3 domain (FnIII-N), and the region containing the C-terminal fibronectin type 3 domain (FnIII-C) were prepared and studied for their binding activities with respect to SH348-1 and SH357-1. As a result, the antibodies SH348-1 and SH357-1 exhibited binding activities with respect to rEPHA2-ECD, rFnIII-NC, and rFnIII-C (Figure 5B). Thus, the antibodies SH348-1 and SH357-1 were shown to bind to a region from amino acids 426 to 534 containing the C-terminal fibronectin type 3 domain (amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing).

(Example 4) In vivo antitumor effect

[0238] MDA-MB-231 cells were dissociated from a culture flask by trypsin treatment and then suspended in 10% FBS-containing RPMI1640 (with antibiotics). After centrifugation, the supernatant was removed. The cells were washed twice with the same medium as above, then suspended in BD Matrigel Basement Membrane Matrix (manufactured by BD Biosciences), and subcutaneously transplanted at a concentration of 5×10^6 cells/mouse into the dorsal region of 6-week-old BALB/cAJcl-nu/nu ((CLEA Japan, Inc.). When the day of transplantation is defined as day 0, SH348-1 or SH357-1 was intraperitoneally administered at a dose of 500 μ g/mouse on days 9, 16, 23, and 30. PBS having the same volume (500 μ l) as that of the antibody was intraperitoneally administered as a control. The tumor volume was measured on days 9, 13, 16, 20, 23, 28, 30, 34, and 37 to study the antitumor effect attributed to the antibody administration. As a result, tumor growth was significantly inhibited in the SH348-1-and SH357-1-administered groups compared to in the PBS-administered group (in the tumor volume comparison with the PBS-administered group on day 37, P values for SH348-1 and SH357-1 were both $P < 0.001$; the P values were calculated by Student's t-test). Moreover, the tumor growth inhibitory rate (= $100 - (\text{average of tumor volumes of the antibody-administered group}) / (\text{average of tumor volumes of the PBS-administered group}) \times 100$) on day 37 was 89.5% for SH348-1 and 84.1% for SH357-1. Their very strong antitumor effects were observed in vivo (Figures 6A and 6B).

[0239] Of 14 anti-EPHA2 monoclonal antibodies studied for their antitumor effects on the MDA-MB-231 cell-transplanted mice, only the antibodies SH348-1 and SH357-1 binding to FnIII-C were shown to be effective (Table 1).

[0240] These results demonstrated that SH348-1 and SH357-1 are antibodies which recognize the previously unreported epitope (amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing) and exhibit an antitumor effect. Moreover, the region to which SH348-1 or SH357-1 binds was shown to serve as a promising target of anti-tumor monoclonal antibodies targeted for EPHA2.

[Table 1]

Table 1

Antibody	Binding activity to antigen				Inhibitory effect on tumor growth (in vivo)
	rEPHA2-ECD	rFnIII-NC	rFnIII-N	rFnIII-C	
SH 348-1	+	+	-	+	+
SH 357-1	+	+	-	+	+
Ab 57-1	+	+	+	-	-
Ab 65-1	+	-	-	-	-
Ab 96-1	+	-	-	-	-
Ab 100-1	+	-	-	-	-
Ab 105-1	+	-	-	-	-
Ab 106-13	+	-	-	-	-
Ab 136-1	+	-	-	-	-
Ab 148-1	+	-	-	-	-
Ab 151-4	+	-	-	-	-
Ab230-1	+	+	+	-	-
Ab 373-1	+	-	-	-	-

Antibody	Binding activity to antigen				Inhibitory effect on tumor growth (in vivo)
	rEPA2-ECD	rFnIII-NC	rFnIII-N	rFnIII-C	
Ab 382-1	+	-	-	-	-

(Example 5) Identification of SH348-1 and SH357-1 antibody genes

[0241] To determine the heavy and light chain N-terminal amino acid sequences of the mouse anti-human EPHA2 antibodies SH348-1 and SH357-1, an aliquot of a solution containing the SH348-1 or SH357-1 purified in paragraph 2)-8 was separated by SDS-PAGE. The proteins in the gel thus separated were transferred from the gel to a PVDF membrane (0.45 µm in pore size; manufactured by Invitrogen Corp.). The PVDF membrane was washed with a washing buffer (25 mM NaCl, 10 mM sodium borate buffer, pH 8.0), then stained by soaking in a staining solution (50% methanol, 20% acetic acid, 0.05% Coomassie Brilliant Blue) for 5 minutes, and then destained with 90% methanol. Band portions corresponding to the heavy and light chains (heavy chain: the band with smaller mobility, light chain: the band with larger mobility) visualized on the PVDF membrane were excised, and an attempt was made to identify their respective N-terminal amino acid sequences by an automatic Edman method (see Edman, P., et al. (1967) Eur. J. Biochem. 1, 80) using Procise (registered trademark) cLC Protein Sequencer Model 492cLC (Applied Biosystems). For the SH348-1 heavy chain, the amino acid sequence could not be identified by the method. Therefore, the N-terminal pyroglutamic acid was removed using Pfu Pyroglutamate Aminopeptidase (manufactured by TAKARA BIO INC.), and the same procedure as above was then performed to identify an amino acid sequence starting at the second amino acid from the N-terminus.

[0242] As a result, the amino acid sequence (starting at the second amino acid from the N-terminus) of the band corresponding to the SH348-1 heavy chain was

I-Q-L-V-Q-S-G-P (SEQ ID NO: 26 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH348-1 light chain was

D-V-L-M-T-Q-S-P-L-S-L (SEQ ID NO: 27 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH357-1 heavy chain was

Q-I-Q-L-V-Q-S-G-P (SEQ ID NO: 28 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH357-1 light chain was

D-V-L-M-T-Q-T-P-L-S-L-P-V-S-L-G-D-Q-A (SEQ ID NO: 29 in the sequence listing).

These amino acid sequences were compared with antibody amino acid sequence database prepared by Kabat et al. (see Kabat, E.A. et al., (1991) in Sequences of Proteins of Immunological Interest Vol. I and II, U.S. Department of Health and Human Services). As a result, the subtype of the SH348-1 heavy chain (γ2a chain) was miscellaneous, and the subtype of the light chain was kappa light II. Moreover, the subtype of the SH357-1 heavy chain (γ2a chain) was determined to be miscellaneous, and the subtype of the light chain was determined to be kappa light II.

[0243] Thus, the following oligonucleotide primers were synthesized, which respectively hybridized to the 5'-terminal region of an antibody gene coding region belonging to these mouse subtypes and the 3'-terminal region thereof containing a stop codon (see Kabat et al., *ibid*; Matti Kartinen et al. (1988) 25, 859-865; and Heinrich, G. et al. (1984) J. Exp. Med. 159, p. 417-435):

5'-cagatccagttgggtcagctctggacct-3' (DB3F1: sequence listing sequence ID No. 30)

5'-aagatatctcattaccggaggtccgggagaa-3' (MIG2AEVR1: sequence listing sequence ID No. 31)

5'-aagaattcatgaagttgcctgttagg-3' (MK19EIF1: sequence listing sequence ID No. 32)

5'-aagatatcttaacactcattcctgttgaagct-3' (KEVR1: sequence listing sequence ID No. 33)

[0244] To clone cDNAs encoding the SH348-1 and SH357-1 heavy and light chains, mRNA was prepared from the SH348-1- or SH357-1-producing hybridomas using Quick Prep mRNA Purification Kit (manufactured by GE Healthcare Bio-Sciences Corp.,

#27-9254-01). From each mRNA thus obtained, cDNA encoding each antibody heavy or light chain was amplified using TaKaRa One Step RNA PCR Kit (AMV) (manufactured by TAKARA BIO INC., #RR024A) and the primer set for the heavy chain (combination of DB3F1 and MIG2AEVR1) or the primer set for the light chain (combination of MK19EIF1 and KEVR1). These cDNAs amplified by PCR were cloned using Zero Blunt TOPO PCR Cloning Kit (manufactured by Invitrogen Corp.). Each of the cloned heavy and light chain nucleotide sequences was determined using a gene sequence analyzer ("ABI PRISM 3700 DNA Analyzer; Applied Biosystems" or "Applied Biosystems 3730xl Analyzer; Applied Biosystems"). In the sequencing reaction, GeneAmp 9700 (Applied Biosystems) was used.

[0245] The determined nucleotide sequence of the cDNA encoding the SH348-1 heavy chain is represented by SEQ ID NO: 34 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 35. The nucleotide sequence of the cDNA encoding the SH348-1 light chain is represented by SEQ ID NO: 36 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 37 in the sequence listing. The nucleotide sequence of the cDNA encoding the SH357-1 heavy chain is represented by SEQ ID NO: 38 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 39. The nucleotide sequence of the cDNA encoding the SH357-1 light chain is represented by SEQ ID NO: 40 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 41. Sequences represented by nucleotide Nos. 1 to 27 and 1327 to 1350 of SEQ ID NO: 34, a sequence represented by nucleotide Nos. 637 to 660 of SEQ ID NO: 36, sequences represented by nucleotide Nos. 1 to 27 and 1327 to 1350 of SEQ ID NO: 38, and a sequence represented by nucleotide Nos. 637 to 660 of SEQ ID NO: 40 are sequences derived from the primers.

[0246] Moreover, the amino acid sequences of these heavy and light chains were analyzed by comparison with antibody amino acid sequence database prepared by Kabat et al. (see Kabat, E.A., et al. (1991) in "Sequence of Proteins of Immunological Interest Vol. I and II"; U.S. Department of Health and Human Services). As a result, the SH348-1 heavy chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 120 to 449 thereof as a constant region. Moreover, the SH348-1 light chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 113 to 219 thereof as a constant region.

[0247] The SH357-1 heavy chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 120 to 449 thereof as a constant region. Moreover, the SH357-1 light chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 113 to 219 thereof as a constant region.

[0248] The nucleotide sequence encoding the SH348-1 heavy chain variable region is represented by SEQ ID NO: 42 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 43. The nucleotide sequence encoding the SH348-1 heavy chain constant region is represented by SEQ ID NO: 44, and the amino acid sequence thereof is represented by SEQ ID NO: 45. The nucleotide sequence encoding the SH348-1 light chain variable region is represented by SEQ ID NO: 46 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 47. The nucleotide sequence encoding the SH348-1 light chain constant region is represented by SEQ ID NO: 48, and the amino acid sequence thereof is represented by SEQ ID NO: 49. Sequences represented by nucleotide Nos. 1 to 27 of SEQ ID NO: 42, a sequence represented by nucleotide Nos. 970 to 993 of SEQ ID NO: 44, and sequences represented by nucleotide Nos. 301 to 324 of SEQ ID NO: 48, are sequences derived from the primers.

[0249] Moreover, the nucleotide sequence encoding the SH357-1 heavy chain variable region is represented by SEQ ID NO: 50 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 51. The nucleotide sequence encoding the SH357-1 heavy chain constant region is represented by SEQ ID NO: 52, and the amino acid sequence thereof is represented by SEQ ID NO: 53. The nucleotide sequence encoding the SH357-1 light chain variable region is represented by SEQ ID NO: 54 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 55. The nucleotide sequence encoding the SH357-1 light chain constant region is represented by SEQ ID NO: 56, and the amino acid sequence thereof is represented by SEQ ID NO: 57. Sequences represented by nucleotide Nos. 1 to 27 of SEQ ID NO: 50, a sequence represented by nucleotide Nos. 970 to 993 of SEQ ID NO: 52, and sequences represented by nucleotide Nos. 301 to 324 of SEQ ID NO: 56 are sequences derived from the primers.

[0250] Furthermore, the positions and sequences of CDRs in each of the amino acid sequences of the heavy and light chain variable regions were analyzed and determined by homology comparison with an antibody amino acid sequence database prepared by Kabat et al. (see Kabat, E.A., et al. (1991) *ibid*). According to the document, even different antibodies have

framework regions which have an amino acid length almost equal to each other and are observed to have amino acid sequence commonality in the variable regions, if they are in the same subtype. On the other hand, CDRs are specific sequences flanked by these framework regions. Thus, the amino acid sequences of the heavy and light chains were analyzed by comparison with those of the same subtype thereas. As a result, CDRs in the SH348-1 heavy chain were determined to have amino acid sequences represented by amino acid Nos. 26 to 35 of SEQ ID NO: 35 in the sequence listing (CDRH₁), amino acid Nos. 50 to 66 thereof (CDRH₂), and amino acid Nos. 99 to 108 thereof (CDRH₃). CDRs in the SH348-1 light chain were determined to have amino acid sequences represented by amino acid Nos. 24 to 39 of SEQ ID NO: 37 in the sequence listing (CDRL₁), amino acid Nos. 55 to 61 thereof (CDRL₂), and amino acid Nos. 94 to 102 thereof (CDRL₃). CDRs in the SH357-1 heavy chain were determined to have amino acid sequences represented by amino acid Nos. 26 to 35 of SEQ ID NO: 39 in the sequence listing (CDRH₁), amino acid Nos. 50 to 66 thereof (CDRH₂), and amino acid Nos. 99 to 108 thereof (CDRH₃). CDRs in the SH357-1 light chain were determined to have amino acid sequences represented by amino acid Nos. 24 to 39 of SEQ ID NO: 41 in the sequence listing (CDRL₁), amino acid Nos. 55 to 61 thereof (CDRL₂), and amino acid Nos. 94 to 102 thereof (CDRL₃).

[0251] The nucleotide sequence encoding the SH348-1 CDRH₁ is represented by SEQ ID NO: 58 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 59. The nucleotide sequence encoding the SH348-1 CDRH₂ is represented by SEQ ID NO: 60, and the amino acid sequence thereof is represented by SEQ ID NO: 61. The nucleotide sequence encoding the SH348-1 CDRH₃ is represented by SEQ ID NO: 62, and the amino acid sequence thereof is represented by SEQ ID NO: 63. The nucleotide sequence encoding the SH348-1 CDRL₁ is represented by SEQ ID NO: 64, and the amino acid sequence thereof is represented by SEQ ID NO: 65. The nucleotide sequence encoding the SH348-1 CDRL₂ is represented by SEQ ID NO: 66, and the amino acid sequence thereof is represented by SEQ ID NO: 67. The nucleotide sequence encoding the SH348-1 CDRL₃ is represented by SEQ ID NO: 68, and the amino acid sequence thereof is represented by SEQ ID NO: 69. Moreover, the nucleotide sequence encoding the SH357-1 CDRH₁ is represented by SEQ ID NO: 70, and the amino acid sequence thereof is represented by SEQ ID NO: 71. The nucleotide sequence encoding the SH357-1 CDRH₂ is represented by SEQ ID NO: 72, and the amino acid sequence thereof is represented by SEQ ID NO: 73. The nucleotide sequence encoding the SH357-1 CDRH₃ is represented by SEQ ID NO: 74, and the amino acid sequence thereof is represented by SEQ ID NO: 75. The nucleotide sequence encoding the SH357-1 CDRL₁ is represented by SEQ ID NO: 76, and the amino acid sequence thereof is represented by SEQ ID NO: 77. The nucleotide sequence encoding the SH357-1 CDRL₂ is represented by SEQ ID NO: 78, and the amino acid sequence thereof is represented by SEQ ID NO: 79. The nucleotide sequence encoding the SH357-1 CDRL₃ is represented by SEQ ID NO: 80, and the amino acid sequence thereof is represented by SEQ ID NO: 81.

(Example 6) Binding activity of anti-EPHA2 antibody to EPHA2 extracellular region

[0252] A solution of an EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100) or bovine serum albumin (in the description below and the figures, abbreviated to "BSA") as a control diluted to 1 µg/ml with PBS was dispensed at 100 µl/well onto an immunoplate (manufactured by Nunc, #442404) and incubated overnight at 4°C to thereby adsorb the protein to the plate.

[0253] On the next day, the solution in the wells was removed, and a Block Ace solution (one pouch of Block Ace powder (manufactured by Dainippon Sumitomo Pharma Co., Ltd. (Show Brand Milk Products Co., Ltd.)) was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS was dispensed at 200 µl/well and incubated at room temperature for 1 hour. The wells were washed twice with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, SH348-1, SH357-1, and Ab96-1 were separately diluted with PBS to a concentration of 1.25×10^{-4} µg/ml, 1.25×10^{-3} µg/ml, 1.25×10^{-2} µg/ml, 1.25×10^{-1} µg/ml, 1.25 µg/ml, 12.5 µg/ml, or 125 µg/ml, and the resulting solution (containing 0.05% (v/v) (final concentration) Tween 20) was added at 100 µl/well.

[0254] The plate was incubated at room temperature for 1 hour. Then, the solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Goat anti-Mouse IgG, Peroxidase Conjugated (manufactured by Millipore (Chemicon), #AP181P) diluted 1000 times with Diluting Buffer was added at 100 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was performed with stirring by the addition of OPD Color Developing Solution at 100 µl/well. After color development, the color reaction was terminated by the addition of 1 M HCl at 100 µl/well. The absorbance at 490 nm was measured using a plate reader (Figure 7).

[0255] Figure 7A) shows the results of SH348-1, Figure 7B) shows the results of SH357-1, and Figure 7C) shows the results of Ab96-1. In each graph, the absorbance is indicated in mean±standard deviation (n = 3). Stronger absorbance represents

stronger binding activity. As shown in the graphs, all the antibodies SH348-1, SH357-1, and Ab96-1 exhibited no affinity for BSA, demonstrating that they specifically bind to the EPHA2 extracellular region.

(Example 7) Influence of anti-EPHA2 antibody on ligand binding

[0256] An EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100)-immobilized immunoplate was prepared according to the method described in Example 6. The immunoplate wells were washed twice with Diluting Buffer. Then, SH348-1, SH357-1, Ab96-1, or Mouse IgG_{2A} Isotype Control (in the description below and the figures, abbreviated to "mIgG2a"; manufactured by R&D Systems, Inc., #MAB003) as an isotype control antibody diluted to 10 µg/ml or 50 µg/ml with Diluting Buffer was added at 100 µl/well. The plate was incubated at room temperature for 1 hour. Then, Recombinant Mouse Ephrin-A1/Fc Chimera (in the description below and the figures, abbreviated to "Ephrin-A1/Fc"; manufactured by R&D Systems, Inc., #602-A1-200) as a soluble ligand or Recombinant Human IgG₁ Fc (in the description below and the figures, abbreviated to "hG₁Fc"; manufactured by R&D Systems, Inc., #110-HG-100) as a negative control protein for the soluble ligand was added at a final concentration of 1 µg/ml and incubated at room temperature for 1 hour.

[0257] Next, according to the method described in Example 6, the solution in the wells was removed, and the wells were washed with Diluting Buffer. Peroxidase AffiniPure Goat Anti-Human IgG Fcγ Fragment Specific (Jackson ImmunoResearch Laboratories, Inc., #109-035-098) was added thereto. A color reaction was performed using OPD Color Developing Solution. The absorbance at 490 nm was measured using a plate reader (Figure 8).

[0258] In Figure 8, the absorbance is indicated in mean±standard deviation (n = 3). The antibody Ab96-1 even at a concentration of 10 µg/ml strongly inhibited the binding of the EPHA2 ligand ephrin-A1 to EPHA2. By contrast, the antibodies SH348-1 and SH357-1, even when added at a concentration of 50 µg/ml (five times the concentration of Ab96-1), did not inhibit the binding of Ephrin-A1/Fc to EPHA2. These results demonstrated that the antibodies SH348-1 and SH357-1 do not inhibit the binding of Ephrin-A1/Fc to EPHA2.

(Example 8) Verification of inhibitory activity of anti-EPHA2 antibody against ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues

8)-1 Preparation of cell lysates

[0259] MDA-MB-231 cells suspended in RPMI1640 containing 10% FBS, 50 units/ml penicillin, and 50 µg/ml streptomycin (hereinafter, abbreviated to "10% FBS-containing RPMI1640 (with antibiotics)") were seeded at 2.5×10^5 cells/well onto a 12-well dish and cultured overnight at 37°C in 5% CO₂. Next, the medium in the wells was discarded, and RPMI1640 was newly added thereto. The cells were further cultured overnight at 37°C in 5% CO₂. Next, the medium in the wells was removed, and only RPMI1640 or RPMI1640 containing the antibody (mIgG2a, SH348-1, or SH357-1) at a concentration of 10 µg/ml or 50 µg/ml was added to each well and preincubated at 37°C in 5% CO₂ for 1 hour.

[0260] To the thus-preincubated wells supplemented with only RPMI1640, a 1/50 volume of Ephrin-A1/Fc or hG₁Fc (final concentration of 1 µg/ml) or RPMI1640 with the same volume thereof (in Figure 9, represented by (-)) were added. Moreover, to the wells supplemented with mIgG2a, SH348-1, or SH357-1, a 1/50 volume of Ephrin-A1/Fc (final concentration of 1 µg/ml) was added.

[0261] The dishes were further incubated at 37°C in 5% CO₂ for 15 minutes. After discarding of the supernatants, 1×cell Lysis Buffer (manufactured by Cell Signaling Technology, Inc.) containing 1 mM PMSF (manufactured by Sigma-Aldrich, Inc.) and Protease Inhibitor Cocktail (manufactured by Sigma-Aldrich, Inc., #P8340) (hereinafter, abbreviated to PPCLB) was added thereto to lyse the cells. The lysates were centrifuged at 15000 rpm for 5 minutes, and the obtained supernatants were used as immunoprecipitation samples. The protein concentrations of the samples were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

8)-2 Detection of phosphorylated state of EPHA2 by immunoprecipitation

[0262] 25 µl of a suspension of Protein G magnetic beads (manufactured by NEW ENGLAND BioLabs, Inc.) and 4 µg of anti-EphA2 Antibody (manufactured by Santa Cruz Biotechnology, Inc., #sc-924) were added per sample, and the mixture was inverted for mixing at 4°C for 2 hours. Next, FBS was added thereto at a final concentration of 10%, and the mixture was further inverted for mixing at 4°C for 30 minutes. Next, the beads were washed with PPCLB. Next, 200 µg of the cell lysate supernatants prepared in paragraph 8)-1 were added thereto, and the mixtures were inverted for mixing overnight at 4°C.

[0263] On the next day, the beads were washed three times with PPCLB. Then, SDS-Sample Buffer (56.3 mM Tris-HCl, pH 6.8, 1.8% (w/v) SDS, 9% glycerol, 0.72 M 2-mercaptoethanol, 0.045 mg/ml bromophenol blue) was added to the beads, and the mixtures were heated at 98°C for 5 minutes. The proteins dissociated from the beads were separated by SDS-PAGE.

[0264] The proteins were transferred from the gel to a PVDF membrane (0.45 µm in pore size; manufactured by Millipore). The PVDF membrane was blocked by shaking in sodium azide-free Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 was then added at a final concentration of 0.1% (v/v)).

[0265] To detect the phosphorylated state of the EPFA2 tyrosine residues, the PVDF membrane was soaked in a solution of Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (manufactured by Upstate, #16-184) diluted 10000 times with sodium azide-free Blocking Solution, and reacted at room temperature for 1 hour. The PVDF membrane was washed for 10 minutes three times with TBST (50 mM Tris-HCl, pH 8.0, 138 mM NaCl, 2.7 mM KCl, 0.1% (v/v) Tween 20) and then further washed for 5 minutes three times with H₂O. Signals were detected on a film for chemiluminescence using ECL Plus (manufactured by GE Healthcare Bio-Sciences Corp.).

[0266] Next, to detect the immunoprecipitated EPFA2 present on this PVDF membrane, the PVDF membrane after the detection of the phosphorylated state of the EPFA2 tyrosine residues was soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol) and shaken at 55°C for 30 minutes. Then, the PVDF membrane was soaked in Quenching Solution (TBST containing 1% (v/v) H₂O₂ and 0.1% (w/v) NaN₃) and shaken at room temperature for 20 minutes to remove the antibody on the PVDF membrane. The PVDF membrane was washed for 10 minutes three times with TBST and blocked in Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). Then, the PVDF membrane was soaked in a solution of an anti-EphA2 Antibody (manufactured by Santa Cruz Biotechnology, Inc., #sc-924) diluted 4000 times with Blocking Solution, and reacted at room temperature for 1 hour. The PVDF membrane was washed for 10 minutes three times with TBST. Then, the PVDF membrane was soaked in a solution of Anti-rabbit IgG, HRP-linked Antibody (manufactured by Cell Signaling Technology, Inc., #7074) diluted 10000 times with TBST, and reacted at room temperature for 30 minutes. Then, this PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

[0267] The soluble ligand Ephrin-A1/Fc-induced phosphorylation of EPFA2 tyrosine residues was not inhibited by the isotype control antibody mlgG2a but was inhibited in a dose-dependent manner by the addition of the antibodies SH348-1 and SH357-1 (Figure 9).

[0268] These results demonstrated that the antibodies SH348-1 and SH357-1 do not inhibit the binding of the ligand ephrin-A1 to EPFA2 but inhibit the ligand-induced phosphorylation of EPFA2 tyrosine residues.

(Example 9) Epitope identification for anti-EPFA2 antibody

[0269] Deletion mutants of EPFA2 consisting of a region shown in Figure 10 were prepared, and a region binding to SH348-1 or SH357-1 was determined.

9)-1 Preparation of deletion mutants of EPFA2

[0270] To express deletion mutants of EPFA2 as proteins having GST-Tag and His-Tag added to the N-terminus and S-Tag added to the C-terminus, the following primers were synthesized, and gene fragments amplified by a method shown below were cloned into pET-49b (+) (manufactured by Novagen):

5'-attagatccgagcttccgtactgccagtgtc-3' (primer N1: sequence ID No. 82)

5'-attagatccgcccccaaggtaggct-3' (primer N2: sequence ID No. 83)

5'-attagatccggtcactaccgcaagaaggaga-3' (primer N3: sequence ID No. 84)

5'-attagatccggtccagggtcaggcactgacg-3' (primer N4: sequence ID No. 85)

5'-aattaagcttgccgccaatcaccgccaagtt-3' (primer C1: sequence ID No. 86)

5'-aattaagcttggtccagatccctccggggac-3' (primer C2: sequence ID No. 87)

5'-aattaagcttcaggtaggtggtctctggg-3' (primer C3: sequence ID No. 88)

5'-aattaagcttctgtactccacactcggc-3' (primer C4: sequence ID No. 89)

[0271] Each region was amplified by PCR reaction using pcDNA-DEST40-EPHA2 as the template and a primer set: the primers N1 and C1 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 540 of SEQ ID NO: 8 in the sequence listing (hereinafter, referred to as "m1"); the primers N1 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 thereof (hereinafter, referred to as "m2"); the primers N1 and C3 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 504 thereof (hereinafter, referred to as "m3"); the primers N1 and C4 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 470 thereof (hereinafter, referred to as "m4"); the primers N2 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 thereof (hereinafter, referred to as "m5"); the primers N3 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 471 to 534 thereof (hereinafter, referred to as "m6"); the primers N4 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 505 to 534 thereof (hereinafter, referred to as "m7"); the primers N2 and C3 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 504 thereof (hereinafter, referred to as "m8"); and the primers N2 and C4 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 470 thereof (hereinafter, referred to as "m9"). The obtained PCR products were cleaved with BamHI and HindIII and subcloned into the BamHI/HindIII site of pET-49b (+).

9)-2 Expression of deletion mutants of EPHA2

[0272] Escherichia coli BL21 (DE3) was transformed with the plasmid DNA constructed in paragraph 9)-1 or pET-49b (+) as a negative control. The obtained transformants were cultured in an LB medium supplemented with 30 µg/ml kanamycin (manufactured by Invitrogen Corp.). Expression of the deletion mutants of EPHA2 was induced using Autoinduction System (manufactured by Novagen). The bacterial cells were collected by centrifugation and washed with PBS. Then, the bacterial cells were lysed with a 2% SDS solution containing 1 mM PMSF and Protease Inhibitor Cocktail (manufactured by Sigma-Aldrich, Inc., #P8340). The supernatant was collected by centrifugation and used in epitope identification.

[0273] The expression level of proteins (consisting of pET-49b (+)-derived GST-Tag, His-Tag, and S-Tag and linker portions for connecting them; in the description below and the figures, referred to as "Vec") expressed in the bacterial cell lysate of Escherichia coli transformed with pET-49b (+) was estimated by a method described below. Dilution series of the Vec-expressing bacterial cell lysate and dilution series of 6x His Protein Ladder (manufactured by QIAGEN) were separately dissolved in SDS-Sample Buffer and heated at 98°C for 5 minutes. Then, the proteins were separated by SDS-PAGE, and the proteins in the gel were transferred to a PVDF membrane. The PVDF membrane was blocked in TBST containing 5% BSA. Then, the PVDF membrane was soaked in a solution of Penta-His HRP Conjugate (manufactured by QIAGEN) diluted 1000 times with TBST containing 5% BSA, and reacted at room temperature for 1 hour. This PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected using ECL Plus. Signal intensity was compared between the dilution series of the Vec-expressing bacterial cell lysate and the dilution series of 6x His Protein Ladder, and the concentration of the Vec protein contained in the Vec-expressing bacterial cell lysate was estimated from the protein level per band contained in 6x His Protein Ladder. Deletion mutants of EPHA2 with an amount that exhibited reactivity equivalent to that of 20 ng of Vec in Western blotting using S-Tag Monoclonal Antibody (Novagen) were used in the subsequent epitope identification experiment.

9)-3) Epitope identification

[0274] The cell lysates containing the deletion mutants of EPHA2 prepared in paragraph 9)-2 were dissolved in SDS-Sample

Buffer and heated at 98°C for 5 minutes. The resulting samples were separated by SDS-PAGE, and the proteins in the gels were transferred to PVDF membranes. After the transfer, the PVDF membranes were blocked by shaking in Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). Then, these PVDF membranes were reacted overnight at 4°C in Blocking Solution containing 2 µg/ml SH348-1 or SH357-1. These PVDF membranes were washed for 10 minutes three times with TBST and further reacted at room temperature for 30 minutes in a solution of Anti-Mouse Ig, HRP-Linked Whole Ab Sheep diluted 5000 times with TBST. Subsequently, the PVDF membranes were washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

[0275] Next, the PVDF membranes were soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol), then shaken at 55°C for 30 minutes, and then washed for 10 minutes three times with TBST. These PVDF membranes were blocked in Blocking Solution and then washed for 10 minutes three times with TBST. The PVDF membranes were soaked in a solution of S-Tag Monoclonal Antibody diluted 10000 times with TBST, and reacted at room temperature for 30 minutes. The PVDF membranes were washed for 10 minutes three times with TBST. Then, the PVDF membranes were soaked in a solution of Anti-Mouse Ig, HRP-Linked Whole Ab Sheep diluted 5000 times with TBST, and reacted at room temperature for 30 minutes. Next, the PVDF membranes were washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

[0276] As a result, both the antibodies SH348-1 (Figure 11A) and SH357-1 (Figure 11C) exhibited binding activities only to m1, m2, and m5. Moreover, in this procedure, the deletion mutants of EPHA2 were present in an almost constant amount on each of the PVDF membranes (SH348-1: Figure 11B, SH357-1: Figure 11D). These results demonstrated that the antibodies SH348-1 and SH357-1 bind to a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing in the EPHA2 amino acid sequence.

(Example 10) Design of humanized antibody

10)-1 Design of humanized antibody of SH348-1

10)-1-1 Molecular modeling of SH348-1 variable regions

[0277] The molecular modeling of SH348-1 variable regions was conducted according to homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences (three-dimensional structures derived from X-ray crystal structures are available) of human immunoglobulin variable regions registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) were compared with the SH348-1 variable regions determined in Example 5. As a result, 2JEL and 1A4J were respectively selected as sequences having the highest sequence homology to the SH348-1 light or heavy chain variable regions. The three-dimensional structures of framework regions were prepared based on a "framework model" obtained by combining the coordinates of 2JEL and 1A4J corresponding to the SH348-1 light and heavy chains. For SH348-1 CDRs, CDRL₁, CDRL₂, CDRL₃, CDRH₁, and CDRH₂ were assigned to clusters 16A, 7A, 9A, 10A, and 10A, respectively, according to the classification of Thornton et al. (J. Mol. Biol., 263, 800-815, (1996)). CDRH₃ was classified in e (9) D according to the H3-rules (FEBS letters 399, 1-8 (1996)). Subsequently, the typical conformation of each CDR was incorporated in the framework model.

[0278] Finally, to obtain possible molecular models of the SH348-1 variable regions in terms of energy, an energy calculation was conducted for excluding disadvantageous interatomic contact. These procedures were performed using a commercially available three-dimensional protein structure prediction program Prime and coordinate search program MacroModel (Schrödinger, LLC).

10)-1-2 Design of amino acid sequence of humanized SH348-1

[0279] Humanized SH348-1 antibodies were constructed according to CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Acceptor antibodies were selected based on amino acid homology within the framework regions. The sequences of the SH348-1 framework regions were compared with those of all human frameworks registered in the Kabat Database (Nuc. Acid Res. 29, 205-206 (2001)) involving antibody amino acid sequences. A GSD2B5B10'CL antibody was selected as an acceptor due to

their having, at 72%, the highest sequence homology between their framework regions. The amino acid residues of the framework regions in GSD2B5B10'CL were aligned with the corresponding amino acid residues in SH348-1 to identify positions where different amino acids therebetween were used. The positions of these residues were analyzed using the three-dimensional model of SH348-1 thus constructed. Then, donor residues to be grafted on the acceptor were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Humanized SH348-1 sequences were determined by transferring some selected donor residues to the acceptor antibody GSD2B5B10'CL. As a result, the humanized sequences of two types of light chains and two types of heavy chains were obtained as shown below. Hereinafter, variable and constant regions and CDRs were classified based on the antibody amino acid sequence database prepared by Kabat et al.

10-1-3) Humanization of SH348-1 light chain

10-1-3-1) hSH348-T1L-type light chain:

[0280] A humanized SH348-1 light chain was designed by substituting amino acid Nos. 2 (valine), 3 (leucine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 79 (arginine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH348-1 light chain variable region shown in SEQ ID NO: 37 of the sequence listing with isoleucine, valine, threonine, proline, glutamic acid, proline, glutamine, lysine, valine, glutamine, valine, and threonine, respectively, and was designated as a "hSH348-T1L-type light chain".

[0281] The nucleotide sequence encoding the hSH348-1-T1L-type light chain is represented by SEQ ID NO: 90 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 91. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 90 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

[0282] The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 91 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

[0283] Moreover, the nucleotide sequence encoding the hSH348-1-T1L-type light chain CDRL₁ is represented by SEQ ID NO: 92 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 93, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 94 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 95, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 96 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 97.

10-1-3-2) hSH348-T3L-type light chain:

[0284] A humanized SH348-1 light chain was designed by substituting amino acid Nos. 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 79 (arginine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH348-1 light chain variable region shown in SEQ ID NO: 37 of the sequence listing with threonine, proline, glutamic acid, proline, glutamine, lysine, valine, glutamine, valine, and threonine, respectively, and was designated as a "hSH348-1-T3L-type light chain".

[0285] The nucleotide sequence encoding the hSH348-1-T3L-type light chain is represented by SEQ ID NO: 98 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 99. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 98 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos.

340 to 363 thereof is CDRL₃.

[0286] The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 99 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

[0287] Moreover, the nucleotide sequence encoding the hSH348-1-T3L-type light chain CDRL₁ is represented by SEQ ID NO: 100 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 101, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 102 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 103, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 104 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 105.

10-1-4) Humanization of SH348-1 heavy chain

10-1-4-1) hSH348-1-T1H-type heavy chain:

[0288] A humanized SH348-1 heavy chain was designed by substituting amino acid Nos. 2 (isoleucine), 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 46 (lysine), 68 (phenylalanine), 69 (alanine), 70 (phenylalanine), 71 (serine), 72 (leucine), 73 (glutamic acid), 76 (alanine), 80 (phenylalanine), 82 (glutamine), 83 (isoleucine), 84 (asparagine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (threonine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH348-1 heavy chain variable region shown in SEQ ID NO: 43 of the sequence listing with valine, alanine, valine, serine, serine, valine, arginine, glutamine, glutamic acid, valine, threonine, isoleucine, threonine, alanine, aspartic acid, threonine, tyrosine, glutamic acid, leucine, serine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH348-1-T1H-type heavy chain".

[0289] The nucleotide sequence encoding the hSH348-1-T1H-type heavy chain is represented by SEQ ID NO: 106 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 107. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 106 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 148 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

[0290] The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 107 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 50 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

[0291] Moreover, the nucleotide sequence encoding the hSH348-1-T1H-type heavy chain CDRH₁ is represented by SEQ ID NO: 108 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 109, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 110 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 111, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 112 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 113.

10-1-4-2) hSH348-1-T3H-type heavy chain:

[0292] A humanized SH348-1 heavy chain was designed by substituting amino acid Nos. 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 73 (glutamic acid), 76 (alanine), 80 (phenylalanine), 82 (glutamine), 83 (isoleucine), 84 (asparagine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (threonine), 95 (phenylalanine), 114

(threonine), and 115 (leucine) of SH348-1 heavy chain variable domains shown in SEQ ID NO: 43 of the sequence listing with alanine, valine, serine, serine, valine, arginine, glutamine, aspartic acid, threonine, tyrosine, glutamic acid, leucine, serine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH348-1-T3H-type heavy chain".

[0293] The nucleotide sequence encoding the hSH348-1-T3H-type heavy chain is represented by SEQ ID NO: 114 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 115. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 114 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 148 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

[0294] The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 115 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 50 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

[0295] Moreover, the nucleotide sequence encoding the hSH348-1-T3H-type heavy chain CDRH₁ is represented by SEQ ID NO: 116 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 117, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 118 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 119, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 120 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 121.

10)-2 Design of humanized antibody of SH357-1

10)-2-1 Molecular modeling of SH357-1 variable region

[0296] 2JEL and 1A4J were selected as sequences having the highest sequence homology to the SH357-1 light and heavy chain variable regions, respectively, in the same way as in paragraph 10)-1-1. CDRL₁, CDRL₂, CDRL₃, CDRH₁, and CDRH₂ were assigned to clusters 16A, 7A, 9A, 10A, and 10A, respectively. CDRH₃ was classified in e(9)D.

10)-2-2 Design of amino acid sequence for humanized SH357-1

[0297] A GSD2B5B10'CL antibody was selected as an acceptor in the same way as in paragraph 10)-1-2, and the sequence of humanized SH357-1 was determined. As a result, the humanized sequences of two types of light chains and two types of heavy chains were obtained as shown below.

10)-2-3) Humanization of SH357-1 light chain

10)-2-3-1) hSH357-1-T1L-type light chain:

[0298] A humanized SH357-1 light chain designed by substituting amino acid Nos. 2 (valine), 3 (leucine), 7 (threonine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH357-1 light chain shown in SEQ ID NO: 41 of the sequence listing with isoleucine, valine, serine, threonine, proline, glutamic acid, proline, glutamine, valine, glutamine, valine, and threonine, respectively, was designated as a "hSH357-1-T1L-type light chain".

[0299] The nucleotide sequence encoding the hSH357-1-T1L-type light chain is represented by SEQ ID NO: 122 in the

sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 123. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 122 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

[0300] The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 123 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

[0301] Moreover, the nucleotide sequence encoding the hSH357-1-T1L-type light chain CDRL₁ is represented by SEQ ID NO: 124 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 125, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 126 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 127, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 128 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 129.

10-2-3-2) hSH357-1-T3L-type light chain:

[0302] A humanized SH357-1 light chain designed by substituting amino acid Nos. 7 (threonine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH357-1 light chain shown in SEQ ID NO: 41 with serine, threonine, proline, glutamic acid, proline, glutamine, valine, glutamine, valine, and threonine, respectively, was designated as a "hSH357-1-T3L-type light chain".

[0303] The nucleotide sequence encoding the hSH357-1-T3L-type light chain is represented by SEQ ID NO: 130 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 131. A nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 130 is a secretion signal sequence. A nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. A nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. A nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. A nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. A nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

[0304] The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 131 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

[0305] Moreover, the nucleotide sequence encoding the hSH357-1-T3L-type light chain CDRL₁ is represented by SEQ ID NO: 132 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 133, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 134 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 135, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 136 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 137.

10-2-4) Humanization of SH357-1 heavy chain

10-2-4-1) hSH357-1-T1H-type heavy chain:

[0306] A humanized SH357-1 heavy chain was designed by substituting amino acid Nos. 2 (isoleucine), 9 (proline), 11 (leucine),

16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 46 (lysine), 68 (phenylalanine), 69 (alanine), 70 (phenylalanine), 71 (serine), 72 (leucine), 73 (glutamic acid), 76 (alanine), 82 (glutamine), 83 (isoleucine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (serine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH357-1 heavy chain variable region shown in SEQ ID NO: 51 of the sequence listing with valine, alanine, valine, alanine, serine, valine, arginine, glutamine, glutamic acid, valine, threonine, isoleucine, threonine, alanine, aspartic acid, threonine, glutamic acid, leucine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH357-1-T1H-type heavy chain".

[0307] The nucleotide sequence encoding the hSH357-1-T1H-type heavy chain is represented by SEQ ID NO: 138 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 139. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 138 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 145 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

[0308] The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 139 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 49 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

[0309] Moreover, the nucleotide sequence encoding the hSH357-1-T1H-type heavy chain CDRH₁ is represented by SEQ ID NO: 140 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 141, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 142 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 143, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 144 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 145.

10-2-4-2) hSH357-1-T3H-type heavy chain:

[0310] A humanized SH357-1 heavy chain was designed by substituting amino acid Nos. 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 73 (glutamic acid), 76 (alanine), 82 (glutamine), 83 (isoleucine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (serine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH357-1 heavy chain variable region shown in SEQ ID NO: 51 with alanine, valine, alanine, serine, valine, arginine, glutamine, aspartic acid, threonine, glutamic acid, leucine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH357-1-T3H-type heavy chain".

[0311] The nucleotide sequence encoding the hSH357-1-T3H-type heavy chain is represented by SEQ ID NO: 146 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 147. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 146 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 145 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

[0312] The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 147 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 49 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

[0313] Moreover, the nucleotide sequence encoding the hSH357-1-T3H-type heavy chain CDRH₁ is represented by SEQ ID NO: 148 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 149, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 150 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 151, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 152 in the sequence listing, and the amino acid

sequence thereof is represented by SEQ ID NO: 153.

(Example 11) Preparation of humanized anti-EPHA2 antibodies

[0314] To measure the activities of the humanized SH348-1 and the humanized SH357-1, plasmids having the heavy and light chains of the humanized anti-EPHA2 antibodies obtained in Example 10 were constructed as shown below.

11)-1 Construction of humanized anti-EPHA2 antibody expression vectors

11)-1-1 Preparation of versatile humanized antibody light chain expression vector (pEF6/KCL)

[0315] A gene encoding a human antibody light chain signal sequence and a human Ig light chain (K chain) constant region, described in SEQ ID NO: 154 in the sequence listing, was synthesized (Invitrogen Corp.; artificial gene synthesis service) and cleaved with restriction enzymes NheI and PmeI. The cleaved DNA fragment was inserted into the NheI/PmeI site of a vector pEF6/V5-HisB (Invitrogen Corp.) to construct a versatile humanized antibody light chain expression vector (pEF6/KCL).

11)-1-2 Preparation of versatile humanized antibody heavy chain expression vector (pEF1/FCCU-1)

[0316] A gene encoding a human antibody heavy chain signal sequence and a human IgG1 constant region, described in SEQ ID NO: 155 in the sequence listing, was synthesized (Invitrogen Corp.; artificial gene synthesis service) and cleaved with restriction enzymes NheI and PmeI. The cleaved DNA fragment was inserted into the NheI/PmeI site of a vector pEF1/myc-HisB (Invitrogen Corp.) to construct a versatile humanized antibody H chain expression vector (pEF1/FCCU-1).

11)-1-3 hSH348-1-T1L and hSH348-1-T3L -type light chain expression vectors

[0317] Each DNA containing a gene encoding a hSH348-1-T1L or hSH348-1-T3L-type light chain variable region represented by SEQ ID NO: 156 and 157 of the sequence listing, fused with a secretion signal was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with restriction enzymes NdeI and BsiWI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody light chain expression (pEF6/KCL) digested in advance with restriction enzymes NdeI and BsiWI to thereby construct hSH348-1-T1L and hSH348-1-T3L-type light chain expression vectors. The obtained expression vectors were designated as "pEF6/KCL/hSH348-1-T1L" and "pEF6/KCL/hSH348-1-T3L", respectively.

11)-1-4 Construction of hSH348-1-T1H and hSH348-1-T3H-type heavy chain expression vectors

[0318] Each DNA containing a gene encoding a hSH348-1-T1H or hSH348-1-T3H-type heavy chain variable region represented by SEQ ID NO: 158 and 159, respectively, of the sequence listing was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with a restriction enzyme BlnI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody H chain expression (pEF1/FCCU-1) digested in advance with a restriction enzyme BlnI to thereby construct hSH348-1-T1H and hSH348-1-T3H-type heavy chain expression vectors. The obtained expression vectors were designated as "pEF1/FCCU/hSH348-1-T1H" and "pEF1/FCCU/hSH348-1-T3H", respectively.

11)-1-5 Construction of hSH357-1-T1L and hSH357-1-T3L-type light chain expression vectors

[0319] Each DNA containing a gene encoding a hSH357-1-T1L or hSH357-1-T3L-type light chain variable region represented by SEQ ID NO: 160 or 161, respectively, of the sequence listing, fused with a secretion signal was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with restriction enzymes NdeI and BsiWI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody L chain expression (pEF6/KCL) digested in advance with restriction enzymes NdeI and BsiWI to thereby construct hSH357-1-T1L and hSH357-1-T3L-type light chain expression

vectors. The obtained expression vectors were designated as "pEF6/KCL/hSH357-1-T1L" and "pEF6/KCL/hSH357-1-T3L", respectively.

11)-1-6 Construction of hSH357-1-T1H and hSH357-1-T3H-type heavy chain expression vectors

[0320] Each DNA containing a gene encoding a hSH357-1-T1H or hSH357-1-T3H-type heavy chain variable region represented by SEQ ID NO: 162 or 163, respectively, of the sequence listing was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with a restriction enzyme BlnI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody H chain expression (pEF1/FCCU-1) digested in advance with a restriction enzyme BlnI to thereby construct hSH357-1-T1H and hSH357-1-T3H-type heavy chain expression vectors. The obtained expression vectors were designated as "pEF1/FCCU/hSH357-1-T1H" and "pEF1/FCCU/hSH357-1-T3H", respectively.

11)-2 Production of humanized antibody

[0321] 1.2×10^9 cells of 293 FreeStyle cells at the log growth phase were seeded onto 1.2 L of fresh FreeStyle 293 Expression Medium (Invitrogen Corp.) and shake-cultured (125 rpm) in an incubator at 37°C in 8% CO₂. 12 mg of Polyethyleneimine (Polyscience #24765) was dissolved in 40 mL of an Opti-Pro SFM medium (manufactured by Invitrogen Corp.) and left at room temperature for 5 minutes. An H chain expression plasmid (0.6 mg) and an L chain expression plasmid (1.8 mg) prepared using a PureLink HiPure Plasmid kit (Invitrogen Corp.) were suspended in 40 mL of an Opti-Pro SFM medium (Invitrogen Corp.). 40 mL of the expression plasmid/OptiPro SFM mixed solution was added to 40 mL of the Polyethyleneimine/OptiPro SFM mixed solution thus left at room temperature for 5 minutes and further left at room temperature for 5 minutes. Next, 80 mL of the Polyethyleneimine/expression plasmid/OptiPro SFM mixed solution was added to the 293 FreeStyle cell suspension, and shake-culture was continued. After 7-day culture at 37°C in 8% CO₂, the culture supernatant was collected.

11)-3 Purification of humanized antibody

[0322] The culture supernatant obtained in paragraph 11)-2 was filtered through a Disposable Capsule Filter (Advantec MFS Inc., #CCS-045-E1H) and then purified by Protein A affinity column chromatography. The culture supernatant was applied to MabSelect SuRe HiTrap 1 mL (manufactured by GE Healthcare Bio-sciences Corp.) equilibrated with PBS, and washed with PBS. Next, a 2 M arginine solution (pH 4.0) was applied thereto to collect antibody-containing fractions. The pH was adjusted to 7, and the antibody-containing fractions were applied to a HiPrep Desalting Column (26/10, 50 mL) (GE Healthcare Bio-sciences Corp.) equilibrated with PBS in advance. After replacement with PBS, the antibody-containing fractions were passed through a 0.2-μm filter to prepare a purified sample.

[0323] The antibody concentration was determined by eluting the antibodies bound to a POROS G 20 μm Column, PEEK, 4.6 mm×100 mm, 1.7 ml (manufactured by Applied Biosystems) and measuring the absorbance (O.D. 280 nm) of the eluate, followed by peak area comparison with a standard (human IgG1).

[0324] A humanized antibody SH348-1 obtained by the combination between pEF6/KCL/hSH348-1-T1L and pEF1/FCCU/hSH348-1-T1H was designated as "hSH348-1-T1"; and a humanized antibody SH348-1 obtained by the combination between pEF6/KCL/hSH348-1-T3L and pEF1/FCCU/hSH348-1-T3H was designated as "hSH348-1-T3".

[0325] Moreover, a humanized antibody SH357-1 obtained by the combination between pEF6/KCL/hSH357-1-T1L and pEF1/FCCU/hSH357-1-T1H was designated as "hSH357-1-T1"; and a humanized antibody SH357-1 obtained by the combination between pEF6/KCL/hSH357-1-T3L and pEF1/FCCU/hSH357-1-T3H was designated as "hSH357-1-T3".

(Example 12) Confirmation of binding activity of humanized anti-EPHA2 antibody to antigen

[0326] The abilities of the antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 to bind to the antigen were confirmed according to the method described in Example 6 except that Peroxidase AffiniPure Goat Anti-Human IgG Fcy Fragment Specific (manufactured by Jackson ImmunoResearch Laboratories, Inc., #109-035-098) was used as a secondary antibody.

[0327] In the graphs of Figures 12A) to 12D), the absorbance is indicated in mean $\pm$ standard deviation (n = 3). As a result, all the humanized anti-EPHA2 antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 were confirmed to have binding activity to the EPHA2 extracellular region.

(Example 13) Measurement of competitive inhibitory activity against binding of SH348-1 or SH357-1 to EPHA2

[0328] The competitive inhibitory activities of hSH348-1-T1 and hSH348-1-T3 against the binding of SH348-1 to EPHA2 as well as the competitive inhibitory activities of hSH357-1-T1 and hSH357-1-T3 against the binding of SH357-1 to EPHA2 were measured by a method described below.

[0329] The mouse monoclonal antibodies SH348-1 and SH357-1 were separately biotinylated using EZ-Link Sulfo-NHS-LC Biotinylation Kit (manufactured by Thermo Fisher Scientific K.K., #21435) according to the protocol included therein (hereinafter, the biotinylated SH348-1 and SH357-1 were referred to as "bSH348-1" and "bSH357-1", respectively). The concentrations of bSH348-1, bSH357-1, and unlabeled antibodies (SH348-1, SH357-1, hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, hSH357-1-T3, and Ab96-1) used in the competitive inhibition experiment were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

[0330] An EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100) was diluted to 0.5 μ g/ml with PBS, then dispensed at 100 μ l/well onto an immunoplate (manufactured by Nunc, #442404), and incubated overnight at 4°C to thereby adsorb the protein onto the plate. On the next day, the wells were washed once with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, a Block Ace solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS was dispensed at 200 μ l/well and incubated at room temperature for 4 hours. The solution in the wells was removed. Then, mixed solutions of the biotinylated antibodies (5 μ g/ml) and various concentrations (0 μ g/ml, 1 μ g/ml, 5 μ g/ml, 25 μ g/ml, 50 μ g/ml, and 125 μ g/ml) of the unlabeled antibodies (solvent: PBS containing 0.05% (v/v) (final concentration) Tween 20) were separately dispensed at 100 μ l/well and incubated at room temperature for 1 hour. The wells were washed twice with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, Streptavidin-horseradish Peroxidase Conjugate (manufactured by GE Healthcare Bio-Sciences Corp., #RPN1231V) diluted 500 times with Diluting Buffer was added at 100 μ l/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was performed with stirring by the addition of OPD Color Developing Solution at 100 μ l/well. After color development, the color reaction was terminated by the addition of 1 M HCl at 100 μ l/well. The absorbance at 490 nm was measured using a plate reader.

[0331] As a result, the absorbance of the wells supplemented with only bSH348-1 or bSH357-1 was 0.780 $\pm$ 0.016 and 0.978 $\pm$ 0.007 (mean $\pm$ standard deviation (n = 3)), respectively.

[0332] In the graphs of Figures 13A) and 13B), the absorbance is indicated in mean $\pm$ standard deviation (n = 3). The binding of SH348-1 or SH357-1 to EPHA2 was not inhibited by Ab96-1 differing in epitope therefrom. On the other hand, the binding of SH348-1 to EPHA2 was shown to be inhibited by the antibody SH348-1 itself or its humanized antibodies hSH348-1-T1 and hSH348-1-T3 (Figure 13A). Likewise the binding of SH357-1 to EPHA2 was shown to be inhibited by the antibody SH357-1 itself or its humanized antibodies hSH357-1-T1 and hSH357-1-T3 (Figure 13B).

(Example 14) Inhibitory effect of humanized anti-EPHA2 antibody on ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues

[0333] The ability of the humanized anti-EPHA2 antibody to inhibit ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues in an ephrin-A1-dependent manner was examined according to the method described in Example 8. As a result, all the antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 were shown to maintain an activity of inhibiting Ephrin-A1/Fc-induced phosphorylation of EPHA2 tyrosine residues (Figure 14).

Industrial Applicability

[0334] An anti-EPHA2 antibody of the present invention has an antitumor activity. A pharmaceutical composition comprising the anti-EPHA2 antibody can be used as an anticancer agent.

SEQUENCE LISTING

[0335]

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<120> Anti EPHA2 antibodies

<130> DSPCT-FP0831

<150> JP 2007-224007

<151> 2007-08-30

<160> 163

<170> PatentIn version 3.4

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cgg gcc atc gca gct gcc atg aag tac ctg gcc aac atg aac tat gtg	2208	
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cac cgt gac ctg gct gcc cgc aac atc ctg gtc aac agc aac atg gtc	2256	
His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val		
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Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro		
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Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr		
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Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile		
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Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr		
945 950 955 960		
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tga	2931	

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 <212> PRT

<213> Homo sapiens

<400> 2

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Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile
50          55          60

Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp
65          70          75          80

Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile
85          90          95

Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala
100          105          110

Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
115          120          125

Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr
130          135          140

Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His
145          150          155          160

Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys
165          170          175

Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu
180          185          190

Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu
195          200          205

Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala
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Thr Val Ala Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly
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Glu Glu Pro Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro
245          250          255

Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala
260          265          270

Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser
275          280          285

Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala
290          295          300

Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro
305          310          315          320

Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr
325          330          335

Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln
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 Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg
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 Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser
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 Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn
 405 410 415
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 420 425 430
 Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser
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 Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser
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 Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn
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 Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp
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 Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln
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 Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu Phe Gln Thr Leu Ser
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 Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly
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 Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
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 Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
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Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
 595 600 605
 His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
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 Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
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 Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
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 675 680 685
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 Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
 705 710 715 720
 Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
 725 730 735
 His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
 740 745 750
 Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
 755 760 765
 Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
 770 775 780
 Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
 785 790 795 800
 Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
 805 810 815
 Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
 820 825 830

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
835 840 845

Leu Met Met Cln Cys Trp Gln Cln Glu Arg Ala Arg Arg Pro Lys Phe
850 855 860

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
865 870 875 880

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
885 890 895

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
900 905 910

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
915 920 925

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
930 935 940

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
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Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
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<213> Artificial Sequence

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<223> Primer 3

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<400> 6

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Ala Cys Phe Ala Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala Ala Ala	
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Ala Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly	
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Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met	
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Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn	
60 65 70	
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Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr	

75	80	85	
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gca gcc tac gag aag gtg gag gat gcc tgc cag gcc tgc tgc cct gga Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly 265 270 275			869
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His Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro
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Gln Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys
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Val Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu
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Pro Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp
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 35 40 45
 Gly Lys Gly Trp Asp Leu Met Cln Asn Ile Met Asn Asp Met Pro Ile
 50 55 60
 Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp
 65 70 75 80
 Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile
 85 90 95
 Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala
 100 105 110
 Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
 115 120 125
 Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr
 130 135 140
 Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His
 145 150 155 160
 Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys
 165 170 175
 Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu
 180 185 190
 Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu
 195 200 205
 Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala
 210 215 220
 Thr Val Ala Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly
 225 230 235 240
 Glu Glu Pro Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro
 245 250 255
 Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala
 260 265 270

Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser
 275 280 285
 Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala
 290 295 300
 Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Cln Asp Pro
 305 310 315 320
 Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr
 325 330 335
 Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln
 340 345 350
 Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln
 355 360 365
 Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg
 370 375 380
 Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser
 385 390 395 400
 Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn
 405 410 415
 Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val
 420 425 430
 Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser
 435 440 445
 Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser
 450 455 460
 Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn
 465 470 475 480
 Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp
 485 490 495
 Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln
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 Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu Phe Gln Thr Leu Ser

515 520 525
 Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly
 530 535 540
 Val Val Leu Leu Leu Val Leu Ala Gly Val Gly Phe Phe Ile His Arg
 545 550 555 560
 Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
 565 570 575
 Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
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 Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
 595 600 605
 His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
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 Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
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 Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
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 His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
 675 680 685
 Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
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Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
770 775 780

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
785 790 795 800

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
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Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
820 825 830

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
835 840 845

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
850 855 860

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
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Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
885 890 895

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
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Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
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Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
930 935 940

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
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gcc gcc gtg gaa gaa acg cta atg gac tcc act aca gcg act gct gag Ala Ala Val Glu Glu Thr Leu Met Asp Ser Thr Thr Ala Thr Ala Glu 20 25 30	96
ctg gcc tgg atg gtg cat cct cca tca ggg tgg gaa gag gtg agt ggc Leu Gly Trp Met Val His Pro Pro Ser Gly Trp Glu Glu Val Ser Gly 35 40 45	144
tac gat gag aac atg aac acg atc cgc acg tac cag gtg tgc aac gtg Tyr Asp Glu Asn Met Asn Thr Ile Arg Thr Tyr Gln Val Cys Asn Val 50 55 60	192
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ctc tat tac tat gag gct gac ttt gac tgc gcc acc aag acc ttc ccc Leu Tyr Tyr Tyr Glu Ala Asp Phe Asp Ser Ala Thr Lys Thr Phe Pro 115 120 125	384
aac tgg atg gag aat cca tgg gtg aag gtg gat acc att gca gcc gac Asn Trp Met Glu Asn Pro Trp Val Lys Val Asp Thr Ile Ala Ala Asp 130 135 140	432
gag agc ttc tcc cag gtg gac ctg ggt gcc cgc gtc atg aaa atc aac Glu Ser Phe Ser Gln Val Asp Leu Gly Gly Arg Val Met Lys Ile Asn 145 150 155 160	480
acc gag gtg cgg agc ttc gga cct gtg tcc cgc agc gcc ttc tac ctg Thr Glu Val Arg Ser Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu 165 170 175	528
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ttc tac cgc aag tgc ccc cgc atc atc cag aat gcc gcc atc ttc cag Phe Tyr Arg Lys Cys Pro Arg Ile Ile Gln Asn Gly Ala Ile Phe Gln 195 200 205	624
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agc tgc atc gcc aat gcg gaa gag gtg gat gta ccc atc aag ctc tac	720

Ser Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr 225 230 235 240	
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465	470	475	480	
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Tyr Asn Ala Thr Ala Ile Lys Ser Pro Thr Asn Thr Val Thr Val Gln				
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Gly Leu Lys Ala Gly Ala Ile Tyr Val Phe Gln Val Arg Ala Arg Thr				
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gtg gca ggc tac ggg cgc tac agc gcc aag atg tac ttc cag acc atg				1584
Val Ala Gly Tyr Gly Arg Tyr Ser Gly Lys Met Tyr Phe Gln Thr Met				
515	520	525		
aca gaa gcc gag tac cag aca agc atc cag gag aag ttg cca ctc atc				1632
Thr Glu Ala Glu Tyr Gln Thr Ser Ile Gln Glu Lys Leu Pro Leu Ile				
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atc gcc tcc tcg gcc gct ggc ctg gtc ttc ctc att gct gtg gtt gtc				1680
Ile Gly Ser Ser Ala Ala Gly Leu Val Phe Leu Ile Ala Val Val Val				
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Ile Ala Ile Val Cys Asn Arg Arg Gly Phe Glu Arg Ala Asp Ser Glu				
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Tyr Thr Asp Lys Leu Gln His Tyr Thr Ser Gly His Met Thr Pro Gly				
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Met Lys Ile Tyr Ile Asp Pro Phe Thr Tyr Glu Asp Pro Asn Glu Ala				
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Val Arg Glu Phe Ala Lys Glu Ile Asp Ile Ser Cys Val Lys Ile Glu				
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Gln Val Ile Gly Ala Gly Glu Phe Gly Glu Val Cys Ser Gly His Leu				
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aag ctg cca ggc aag aga gag atc ttt gtg gcc atc aag acg ctc aag				1968
Lys Leu Pro Gly Lys Arg Glu Ile Phe Val Ala Ile Lys Thr Leu Lys				
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tcg gcc tac acg gag aag cag cgc cgg gac ttc ctg agc gaa gcc tcc				2016
Ser Gly Tyr Thr Glu Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser				
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atc atg ggc cag ttc gac cat ccc aac gtc atc cac ctg gag ggt gtc				2064
Ile Met Gly Gln Phe Asp His Pro Asn Val Ile His Leu Glu Gly Val				
675	680	685		
gtg acc aag agc aca cct gtg atg atc atc acc gag ttc atg gag aat				2112
Val Thr Lys Ser Thr Pro Val Met Ile Ile Thr Glu Phe Met Glu Asn				
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Gly Ser Leu Asp Ser Phe Leu Arg Gln Asn Asp Gly Gln Phe Thr Val				
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 770 775 780
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 Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Gln Tyr
 785 790 795 800
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 Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val Met
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Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr Lys Phe Ile Arg Arg
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Arg Gly Ala His Arg Ile His Val Glu Met Lys Phe Ser Val Arg Asp
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Cys Ser Ser Ile Pro Ser Val Pro Gly Ser Cys Lys Glu Thr Phe Asn
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Leu Tyr Tyr Tyr Glu Ala Asp Phe Asp Ser Ala Thr Lys Thr Phe Pro
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145 150 155 160

Thr Glu Val Arg Ser Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu
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Ala Phe Gln Asp Tyr Gly Gly Cys Met Ser Leu Ile Ala Val Arg Val

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Ser Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr 225 230 235 240		
Cys Asn Gly Asp Gly Glu Trp Leu Val Pro Ile Gly Arg Cys Met Cys 245 250 255		
Lys Ala Gly Phe Glu Ala Val Glu Asn Gly Thr Val Cys Arg Gly Cys 260 265 270		
Pro Ser Gly Thr Phe Lys Ala Asn Gln Gly Asp Glu Ala Cys Thr His 275 280 285		
Cys Pro Ile Asn Ser Arg Thr Thr Ser Glu Gly Ala Thr Asn Cys Val 290 295 300		
Cys Arg Asn Gly Tyr Tyr Arg Ala Asp Leu Asp Pro Leu Asp Met Pro 305 310 315 320		
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 660 665 670

Ile Met Gly Gln Phe Asp His Pro Asn Val Ile His Leu Glu Gly Val
675 680 685

Val Thr Lys Ser Thr Pro Val Met Ile Ile Thr Glu Phe Met Glu Asn
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Gly Ser Leu Asp Ser Phe Leu Arg Gln Asn Asp Gly Gln Phe Thr Val
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725 730 735

Leu Ala Asp Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
740 745 750

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
755 760 765

Arg Phe Leu Glu Asp Asp Thr Ser Asp Pro Thr Tyr Thr Ser Ala Leu
770 775 780

Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Gln Tyr
785 790 795 800

Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val Met
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Trp Glu Val Met Ser Tyr Gly Glu Arg Pro Tyr Trp Asp Met Thr Asn
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Gln Asp Val Ile Asn Ala Ile Glu Gln Asp Tyr Arg Leu Pro Pro Pro
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850 855 860

Lys Asp Arg Asn His Arg Pro Lys Phe Gly Gln Ile Val Asn Thr Leu
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885 890 895

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930 935 940

Val Ser Gln Met Met Met Glu Asp Ile Leu Arg Val Gly Val Thr Leu
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<223> PCR primer 17

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<212> DNA

<213> Homo sapiens

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 tcttctgggg ccagcgatgc gtggaggaat gccgagtaact gcaggggctc cccagggagt 1680
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 ccttctgcgt ggcctgctgc cccagcgctg tgaacctga cctctcctac atgccatct 1860
 ggaagtttc agatgagag ggcgcctgcc agccttgccc catcaactgc acccactcct 1920
 gtgtggacct ggtgacaag ggtgccccg ccgagcagag agccagccct ctgacgtcca 1980
 tcatctctgc ggtggttggc attctgctgg tcgtggtctt gggggtggtc tttgggatcc 2040
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 ccgagctggt ggaagccgctg acacctagcg gaggatgcc caaccaggcg cagatgcgga 2160
 tcttgaaga gacggagctg aggaaggtga aggtgcttgg atctggcgct tttggcacag 2220
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 gccagccctt cgacaacctc tattactggg accaggaccc accagagcgg ggggctccac 3720
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<210> 26

<211> 8

<212> PRT

<213> Mus musculus

<400> 26

Ile Gln Leu Val Gln Ser Gly Pro
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<210> 27

<211> 11

<212> PRT

<213> Mus musculus

<400> 27

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

<210> 28

<211> 9

<212> PRT

<213> Mus musculus

<400> 28

Gln Ile Gln Leu Val Gln Ser Gly Pro
1 5

<210> 29

<211> 19

<212> PRT

<213> Mus musculus

<400> 29

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly

1 5 10 15

Asp Gln Ala

<210> 30

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer DB3F1

<400> 30

cagatccagt tgggcagtc tggacct 27

<210> 31

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer MIG2AEVR1

<400> 31

aagatatctc atttaccgg agtccgggag aa 32

<210> 32

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer MK19EIF1

<400> 32

aagaatcat gaagtgctt gtagg 26

<210> 33

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer KEVR1

<400> 33

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<210> 34

<211> 1350

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(1350)

<400> 34

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1 5 10 15	
aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc aca cac tat	96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr	
20 25 30	
tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg	144
Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met	
35 40 45	
ggc tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc	192
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe	
50 55 60	
aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt	240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe	
65 70 75 80	
ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt	288
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys	
85 90 95	
gca acc tac tat agg tac gaa aga gac ttt gac tac tgg gcc caa ggc	336
Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly	
100 105 110	
acc act ctc aca gtc tcc tca gcc aaa aca aca gcc cca tcg gtc tat	384
Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr	
115 120 125	
cca ctg gcc cct gtg tgt gga gat aca act gcc tcc tcg gtg act cta	432
Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu	
130 135 140	
gga tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg acc tgg	480
Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp	
145 150 155 160	
aac tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct gtc ctg	528
Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu	
165 170 175	
cag tct gac ctc tac acc ctc agc agc tca gtg act gta acc tcg agc	576
Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser	
180 185 190	
acc tgg ccc agc cag tcc atc acc tgc aat gtg gcc cac ccg gca agc	624
Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser	
195 200 205	
agc acc aag gta gac aag aaa att gag ccc aga ggg ccc aca atc aag	672
Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys	
210 215 220	

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ccc tgt cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt gga cca    720
Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
225                230                235                240

tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg atc tcc    768
Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
                245                250                255

ctg agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag gat gac    816
Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
                260                265                270

cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca    864
Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
                275                280                285

gct cag aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg    912
Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
                290                295                300

gtc agt gcc ctc ccc atc cag cac cag gac tgg atg agt ggc aag gag    960
Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
305                310                315                320

ttc aaa tgc aag gtc aac aac aaa gac ctc cca gcg ccc atc gag aga    1008
Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
                325                330                335

acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta tat gtc    1056
Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
                340                345                350

ttg cct cca cca gaa gaa gag atg act aag aza cag gtc act ctg acc    1104
Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
                355                360                365

tgc atg gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc    1152
Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
                370                375                380

aac aac ggg aaa aca gag cta aac tac aag aac act gaa cca gtc ctg    1200
Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
385                390                395                400

gac tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg gaa aag    1248
Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
                405                410                415

aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc cac gag    1296
Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
                420                425                430

ggg ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt    1344
Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
                435                440                445

aaa tga
Lys
                1350

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<210> 35

<211> 449

<212> PRT

<213> Mus musculus

<400> 35

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1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
20 25 30

Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
115 120 125

Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
130 135 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
145 150 155 160

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
180 185 190

Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
210 215 220

Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
 225 230 235 240

Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
 245 250 255

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

Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
 275 280 285

Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
 290 295 300

Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
 305 310 315 320

Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
 325 330 335

Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
 340 345 350

Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
 355 360 365

Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
 370 375 380

Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
 405 410 415

Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
 420 425 430

Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
 435 440 445

Lys

<210> 36

<211> 660

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(660)

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gat caa gcc tcc atc tot tgc aga tct agt cag agc att gta cat agt Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser 20 25 30	96
aat gga aac acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tot Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser 35 40 45	144
cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro 50 55 60	192
gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc agg atc Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile 65 70 75 80	240
agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly 85 90 95	288
tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 100 105 110	336
cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu 115 120 125	384
cag tta aca tot gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe 130 135 140	432
tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg 145 150 155 160	480
caa aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser 165 170 175	528
acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa Thr Tyr Ser Met Ser Ser Thr Leu Thr Thr Lys Asp Glu Tyr Glu 180 185 190	576
cga cat aac agc tat acc tgt gag gcc act ccc aag aca tca act tca	624
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser 195 200 205	
ccc att gtc aag agc ttc aac agg aat gag tgt taa Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys 210 215	660

<210> 37

<211> 219

<212> PRT

<213> Mus musculus

<400> 37

Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro Val Ser Leu Gly
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 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
 20 25 30
 Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95
 Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110
 Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 115 120 125
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 130 135 140
 Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 145 150 155 160
 Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 180 185 190
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
 195 200 205
 Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 210 215

<210> 38

<211> 1350

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(1350)

<400> 38

cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu 1 5 10 15	48
aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc ata gac tat Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr 20 25 30	96
tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met 35 40 45	144
ggc tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe 50 55 60	192
aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr 65 70 75 80	240
ttg cag atc agc aac etc aaa aat gag gac acg gct tca tat ttc tgt Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys 85 90 95	288
gca acc tac tat agg tac gaa aga gac ttt gac tac tgg gcc caa ggc Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly 100 105 110	336
acc act etc aca gtc tcc tca gcc aaa aca aca gcc cca tcg gtc tat Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr 115 120 125	384
cca ctg gcc cct gtg tgt gga gat aca act ggc tcc tcg gtg act cta Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu 130 135 140	432
gga tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg acc tgg Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp 145 150 155 160	480

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aac tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct gtc ctg      528
Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
165                               170                               175

cag tct gac ctg tac acc ctg agc agc tca g-g act gta acc tgg agc      576
Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
180                               185                               190

acc tgg ccc agc cag tcc atc acc tgc aat g-g gcc cac ccg gca agc      624
Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
195                               200                               205

agc acc aag gtg gac aag aaa att gag ccc aga ggg ccc aca atc aag      672
Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
210                               215                               220

ccc tgt cct cca tgc aaa tgc cca gca cct aac ctg ttg ggt gga cca      720
Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
225                               230                               235                               240

tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctg atg atc tcc      768
Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
245                               250                               255

ctg agc ccc ata gtc aca tgt gtg gtg gtg gac gtg agc gag gat gac      816
Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
260                               265                               270

cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca      864
Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
275                               280                               285

gct cag ana caa acc cat aga gag gat tac aac agt act ctg cgg gtg      912
Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
290                               295                               300

gtc agt gcc ctg ccc atc cag cac cag gac tgg atg agt gcc aag gag      960
Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
305                               310                               315                               320

ttc aaa tgc aag gtc aac aac aaa gac ctg cca gcg ccc atc gag aga      1008
Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
325                               330                               335

acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta tat gtc      1056
Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
340                               345                               350

ttg cct cca cca gaa gaa gag atg act aag aaa cag gtc act ctg acc      1104
Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
355                               360                               365

tgc atg gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc      1152
Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
370                               375                               380

aac aac ggg aaa aca gag cta aac tac aag aac act gaa cca gtc ctg      1200
Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
385                               390                               395                               400

gac tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg gaa aag      1248

Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
405                               410                               415

aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc cac gag      1296
Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
420                               425                               430

ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt      1344
Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
435                               440                               445

aaa tga
Lys
1350

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<210> 39

<211> 449

<212> PRT

<213> Mus musculus

<400> 39

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr
 20 25 30
 Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45
 Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
 50 55 60
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80
 Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
 85 90 95
 Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
 115 120 125
 Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
 130 135 140
 Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
 145 150 155 160

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
180 185 190

Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
210 215 220

Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
245 250 255

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

Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
275 280 285

Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
290 295 300

Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
305 310 315 320

Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
325 330 335

Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
340 345 350

Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
355 360 365

Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
370 375 380

Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
405 410 415

Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
420 425 430

Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
435 440 445

Lys

<210> 40

<211> 660

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(660)

<400> 40

gat gtt ttg atg acc caa act cca ctc tcc ctg cct gtc agt ctt gga Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly 1 5 10 15	48
gat caa gcc tcc atc tct tgc aga tct agt cag agc att gta cat agt Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser 20 25 30	96
agt gga atc acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser 35 40 45	144
cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro 50 55 60	192
gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile 65 70 75 80	240
agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt Ser Arg Val Pro Tyr Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly 85 90 95	288
tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 100 105 110	336
cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu 115 120 125	384
cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe 130 135 140	432
tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg 145 150 155 160	480
caa aat ggc gtc ctg aac agt tgg act gat cag gac agc aza gac agc Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser 165 170 175	528
acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu 180 185 190	576
cga cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser 195 200 205	624
ccc att gtc aag agc ttc aac agg aat gag tgt taa Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys 210 215	660

<210> 41

<211> 219

<212> PRT

<213> Mus musculus

<400> 41

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

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

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

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

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

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

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

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
180 185 190

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
195 200 205

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
210 215

<210> 42

<211> 357

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(357)

<400> 42

cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu 1 5 10 15	48
aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc aca cac tat Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr 20 25 30	96
tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met 35 40 45	144
ggc tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe 50 55 60	192
aag gga egg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe 65 70 75 80	240
ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys 85 90 95	288

gca acc tac tat agg tac gaa aga gac ttt gac tac tgg ggc caa ggc 336
Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

acc act ctc aca gtc tcc tca 357
Thr Thr Leu Thr Val Ser Ser
115

<210> 43

<211> 119

<212> PRT

<213> Mus musculus

<400> 43

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

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
20 25 30

Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 44

<211> 993

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(993)

<400> 44

gcc aaa aca aca gcc cca tcg gtc tat cca ctg gcc cct gtg tgt gga 48
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly

1	5	10	15	
gat aca acc ggc tcc tgc gtg act cta gga tgc ctg gtc aag ggt tat				96
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr	20	25	30	
ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt				144
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser	35	40	45	
ggg gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc				192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu	50	55	60	
agc agc tca gtg act gta acc tgg agc acc tgg ccc agc cag tcc atc				240
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile	65	70	75	80
acc tgc aat gtg gcc cac ccg gca agc agc acc aag gta gac aag aaa				288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys	85	90	95	
att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc				336
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys	100	105	110	
cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca				384
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro	115	120	125	
aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt				432
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys	130	135	140	
gtg gtg gtg gat gtg agc gag gat gac cca gat gtc cag atc agc tgg				480
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp	145	150	155	160
ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga				528
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg	165	170	175	
gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag				576
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln	180	185	190	
cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac				624
His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn	195	200	205	
aaa gac ctc cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg				672
Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly	210	215	220	
tca gta aga gct cca cag gta tat gtc ttg cct cca cca gaa gaa gag				720
Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu	225	230	235	240
atg act aag aaa cag gtc act ctg acc tgc atg gtc aca gac ttc atg				768
Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met	245	250	255	
cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag cta				816
Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu	260	265	270	
aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc				864
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe	275	280	285	
atg tac agc aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat				912
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn	290	295	300	
agc tac tcc tgt tca gtg gtc cac gag ggt ctg cac aat cac cac acg				960
Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr	305	310	315	320
act aag agc ttc tcc cgg act ccg ggt aaa tga				993
Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys	325	330		

<210> 45

<211> 330

<212> PRT

<213> Mus musculus

<400> 45

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

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

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

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

Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65 70 75 80

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

Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110

Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125

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

Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160

Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175

Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195 200 205

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

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

Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
245 250 255

Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
260 265 270

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

Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290 295 300

Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305 310 315 320

Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325 330

<210> 46

<211> 336

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1) .. (336)

<400> 46

gat gtt ttg atg acc caa agt cca ctc tcc ctg cct gtc agt ctt gga	48
Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro Val Ser Leu Gly	
1 5 10 15	
gat caa gcc tcc atc tct tgc aga tct agt cag agc att gta cat agt	96
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser	
20 25 30	
aat gga aac acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct	144
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser	
35 40 45	
cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca	192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro	
50 55 60	
gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc agg atc	240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile	
65 70 75 80	
agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt	288
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly	
85 90 95	
tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa	336
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	
100 105 110	

<210> 47

<211> 112

<212> PRT

<213> Mus musculus

<400> 47

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

<210> 48

<211> 324

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(324)

<400> 48

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egg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag      48
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1          5          10          15

cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc      96
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
          20          25          30

tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga     144
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
          35          40          45

caa aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc     192
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
          50          55          60

acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa     240
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65          70          75          80

cga cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca     288
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
          85          90          95

ccc att gtc aag agc ttc aac agg aat gag tgt taa                      324
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
          100          105

```

<210> 49

<211> 107

<212> PRT

<213> Mus musculus

<400> 49

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Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1          5          10          15

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
          20          25          30

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
          35          40          45

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
          50          55          60

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65          70          75          80

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
          85          90          95

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
          100          105

```

<210> 50

<211> 357

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(357)

<400> 50

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cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag      48
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1           5           10           15

aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc ata gac tat      96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr
          20           25           30

tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg      144
Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
          35           40           45

ggc tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc      192
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
          50           55           60

aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat      240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
          65           70           75           80

ttg cag atc agc aac ctc aaa aat gag gac acg gct tca tat ttc tgt      288
Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
          85           90           95

gca acc tac tat agg tac gaa aga gac ttt gac tac tgg ggc caa ggc      336
Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
          100          105          110

acc act ctc aca gtc tcc tca
Thr Thr Leu Thr Val Ser Ser
          115

```

<210> 51

<211> 119

<212> PRT

<213> Mus musculus

<400> 51

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

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

Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
          35           40           45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
          50           55           60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
          65           70           75           80

Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
          85           90           95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
          100          105          110

Thr Thr Leu Thr Val Ser Ser
          115

```

<210> 52

<211> 993

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(993)

<400> 52

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gcc aaa aca aca gcc cca tgc gtc tat cca ctg gcc cct gtg tgt gga      48
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
1      5      10      15

gat aca act ggc tcc tgc gtg act cta gga tgc ctg gtc aag ggt tat      96
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20     25     30

ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt      144

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

ggg gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc      192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50     55     60

agc agc tca gtg act gta acc tgc agc acc tgg ccc agc cag tcc atc      240
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65     70     75     80

acc tgc aat gtg gcc cac ccg gca agc agc acc aag gtg gac aag aaa      288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85     90     95

att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc      336
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100    105    110

cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca      384
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115    120    125

aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt      432
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
130    135    140

gtg gtg gtg gac gtg agc gag gat gac cca gat gtc cag atc agc tgg      480
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145    150    155    160

ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga      528
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165    170    175

gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag      576
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180    185    190

cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac      624
His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195    200    205

aaa gac ctc cca ggc ccc atc gag aca acc atc tca caa ccc aaa ggg      672
Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
210    215    220

tca gta aga gct cca cag gta tat gtc ttg cct cca cca gaa gaa gag      720
Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
225    230    235    240

atg act aag aaa cag gtc act ctg acc tgc atg gtc aca gac ttc atg      768
Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
245    250    255

cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag cta      816
Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
260    265    270

aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc      864
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
275    280    285

atg tac agc aag ctg aga gtg gaa aag aac tgg gtg gaa aga aat      912
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290    295    300

agc tac tcc tgt tca gtg gtc cac gag ggt ctg cac aat cac cac acg      960
Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305    310    315    320

act aag agc ttc tcc cgg act ccg ggt aaa tga      993
Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325    330

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<210> 53

<211> 330

<212> PRT

<213> Mus musculus

<400> 53

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

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

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

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

Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65 70 75 80

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

Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110

Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125

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

Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160

Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175

Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195 200 205

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

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

Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
245 250 255

Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
260 265 270

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

Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290 295 300

Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305 310 315 320

Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325 330

<210> 54

<211> 336

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(336)

<400> 54

gat gtt ttg atg acc caa act cca ctc tcc ctg cct gtc agt ctt gga	48
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly	
1 5 10 15	
gat caa gcc tcc atc tct tgc aga tct agt cag agc att gta cat agt	96
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser	
20 25 30	
agt gga atc acc tat tta gaa tgg tac ctg cag aaa cca gcc cag tct	144
Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser	
35 40 45	
cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca	192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro	
50 55 60	
gac agg ttc agt gcc agt gga tca ggg aca gat ttc aca ctc aag atc	240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile	
65 70 75 80	
agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt	288
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly	
85 90 95	
tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa	336
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	
100 105 110	

<210> 55

<211> 112

<212> PRT

<213> Mus musculus

<400> 55

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

<210> 56

<211> 324

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(324)

<400> 56

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egg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag      48
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1          5          10          15

cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc      96
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
          20          25          30

tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga     144
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
          35          40          45

caa aat ggc gtc ctg aac agt tgg act gat cag gac ago aaa gac agc     192
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
          50          55          60

acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa     240
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65          70          75          80

cga cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca     288
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
          85          90          95

ccc att gtc aag agc ttc aac agg aat gag tgt taa                      324
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
          100          105

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<210> 57

<211> 107

<212> PRT

<213> Mus musculus

<400> 57

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Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1          5          10          15

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
          20          25          30

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
          35          40          45

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
          50          55          60

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65          70          75          80

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
          85          90          95

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
          100          105

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<210> 58

<211> 30

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(30)

<400> 58

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ggg tat acc ttc aca cac tat tca atg cac      30
Gly Tyr Thr Phe Thr His Tyr Ser Met His
1          5          10

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<210> 59

<211> 10

<212> PRT

<213> Mus musculus

<400> 59

Gly Tyr Thr Phe Thr His Tyr Ser Met His
1 5 10

<210> 60

<211> 51

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(51)

<400> 60

tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc aag 48
Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15

gga 51
Gly

<210> 61

<211> 17

<212> PRT

<213> Mus musculus

<400> 61

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15

Gly

<210> 62

<211> 30

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(30)

<400> 62

tac tat agg tac gaa aga gac ttt gac tac 30
Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 63

<211> 10

<212> PRT

<213> Mus musculus

<400> 63

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 64

<211> 48

<212> DNA

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<220>

<221> CDS

<222> (1)..(48)

<400> 64

aga tct agt cag agc att gta cat agt aat gga aac acc tat tta gaa 48
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 65

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<211> 16
<212> PRT
<213> Mus musculus

<400> 65
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1          5          10          15

<210> 66
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(21)

<400> 66
aaa gtt tcc aac cga ttt tct
Lys Val Ser Asn Arg Phe Ser
1          5
21

<210> 67
<211> 7
<212> PRT
<213> Mus musculus

<400> 67
Lys Val Ser Asn Arg Phe Ser
1          5

<210> 68
<211> 27
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(27)

<400> 68
ttt caa ggt tca cat gtt ccg tac acg
Phe Gln Gly Ser His Val Pro Tyr Thr
1          5
27

<210> 69
<211> 9
<212> PRT
<213> Mus musculus

<400> 69
Phe Gln Gly Ser His Val Pro Tyr Thr
1          5

<210> 70
<211> 30
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(30)

<400> 70
ggg tat acc ttc ata gac tat tca atg cac
Gly Tyr Thr Phe Ile Asp Tyr Ser Met His
1          5          10
30

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<210> 71
 <211> 10
 <212> PRT
 <213> Mus musculus

<400> 71
 Gly Tyr Thr Phe Ile Asp Tyr Ser Met His
 1 5 10

<210> 72
 <211> 51
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(51)

<400> 72
 tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc aag 48
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15
 gga 51
 Gly

<210> 73
 <211> 17
 <212> PRT
 <213> Mus musculus

<400> 73
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15
 Gly

<210> 74
 <211> 30
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(30)

<400> 74
 tac tat agg tac gaa aga gac ttt gac tac 30
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 75
 <211> 10
 <212> PRT
 <213> Mus musculus

<400> 75
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 76
 <211> 48
 <212> DNA
 <213> Mus musculus

<220>
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 <222> (1)..(48)

<400> 76
 aga tct agt cag agc att gta cat agt agt gga atc acc tat tta gaa 48
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 77

<211> 16

<212> PRT

<213> Mus musculus

<400> 77
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 78

<211> 21

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(21)

<400> 78
 aaa gtt tcc aac cga ttt tct 21
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 79

<211> 7

<212> PRT

<213> Mus musculus

<400> 79
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 80

<211> 27

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(27)

<400> 80
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 Phe Gln Gly Ser His Val Pro Tyr Thr
 1 5

<210> 81

<211> 9

<212> PRT

<213> Mus musculus

<400> 81
 Phe Gln Gly Ser His Val Pro Tyr Thr
 1 5

<210> 82

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer N1

<400> 82
attaggatcc gagctccgt actgccagtg tc 32

<210> 83
<211> 28

<212> DNA
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<220>
<223> primer N2

<400> 83
attaggatcc gcccccgaag gtgaggct 28

<210> 84
<211> 34
<212> DNA
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<220>
<223> primer N3

<400> 84
attaggatcc ggtcacttac cgcaagaagg gaga 34

<210> 85
<211> 32
<212> DNA
<213> Artificial

<220>
<223> primer N4

<400> 85
attaggatcc ggtccagggtg caggcactga cg 32

<210> 86
<211> 31
<212> DNA
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<220>
<223> primer C1

<400> 86
aattaagctt gccgccaatc accgccaagt t 31

<210> 87
<211> 32
<212> DNA
<213> Artificial

<220>
<223> primer C2

<400> 87
aattaagctt gttgccagat ccctccgggg ac 32

<210> 88
<211> 29
<212> DNA
<213> Artificial

<220>

<223> primer C3

<400> 88

aattaagctt caggtagggtg gtgtctggg 29

<210> 89

<211> 30

<212> DNA

<213> Artificial

<220>

<223> primer C4

<400> 89

aattaagctt ctgctacttc cacactcggc 30

<210> 90

<211> 720

<212> DNA

<213> Artificial

<220>

<223> hSH-348-T1L

<220>

<221> CDS

<222> (1)..(720)

<220>

<221> sig_peptide

<222> (1)..(60)

<400> 90

atg	gtg	ctg	cag	acc	cag	gtg	ttc	atc	tcg	ctg	ctg	tgg	atc	tcg	48
Met	Val	Leu	Gln	Thr	Gln	Val	Phe	Ile	Ser	Leu	Leu	Leu	Trp	Ile	Ser
1			5					10					15		

ggc	gca	tat	ggc	gac	ata	gtg	atg	acc	cag	agc	ccc	ctg	agc	ctg	ccc	96
Gly	Ala	Tyr	Gly	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	
		20					25					30				

gtg	acc	ccc	ggc	gag	ccc	gcc	tcg	atc	agc	tgc	cgg	agc	agc	cag	agc	144
Val	Thr	Pro	Gly	Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	
		35				40					45					

atc	gtg	cac	agc	aac	ggc	aac	acc	tac	ctg	gag	tgg	tac	ctg	cag	aag	192
Ile	Val	His	Ser	Asn	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	
	50				55						60					

ccc	ggc	cag	agc	ccc	cag	ctg	ctg	atc	tac	aag	gtg	agc	aac	cgg	llc	240
Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	
65					70					75				80		

agc	ggc	gtg	ccc	gac	cgg	ttc	agc	ggc	agc	ggc	agc	ggc	acc	gac	ttc	288
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	

85	90	95	
acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac			336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr			
100	105	110	
tgc ttc cag ggc tcc cac gtg ccc tac acc ttc ggc cag ggc acc aag			384
Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys			
115	120	125	
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc			432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro			
130	135	140	
ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg			480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu			
145	150	155	160
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac			528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp			
165	170	175	
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac			576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp			
180	185	190	
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa			624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys			
195	200	205	
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag			672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln			
210	215	220	
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag			720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys			
225	230	235	

<210> 91

<211> 239

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 91

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1 5 10 15

Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45

Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Cln Ser Pro Cln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
115 120 125

Val Glu Ile Iys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 92

<211> 48

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T1L CDRL1

<220>

<221> CDS

<222> (1)..(48)

<400> 92

cgg agc agc cag agc atc gtg cac agc aac ggc aac acc tac ctg gag 48
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 93

<211> 16

<212> PRT

<213> Artificial <220>

<223> Synthetic Construct

<400> 93

Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 94

<211> 21

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T1L CDRL2

<220>

<221> CDS

<222> (1)..(21)

<400> 94

aag gtg agc aac cgg ttc agc
Lys Val Ser Asn Arg Phe Ser
1 5

21

<210> 95

<211> 7

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 95

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 96

<211> 24

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T1L CDRL3

<220>

<221> CDS

<222> (1)..(24)

<400> 96

ttc cag ggc tcc cac gtg ccc tac
Phe Gln Gly Ser His Val Pro Tyr
1 5

24

<210> 97

<211> 8

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 97

Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 98

<211> 720

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3L

<220>

<221> CDS

<222> (1)..(720)

<220>

<221> sig_peptide

<222> (1)..(60)

<400> 98

atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc	48
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser	
1 5 10 15	
ggc gca tat ggc gac gtg ctg atg acc cag agc ccc ctg agc ctg ccc	96
Gly Ala Tyr Gly Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro	
20 25 30	
gtg acc ccc ggc gag ccc gcc tcc atc agc tgc cgg agc agc cag agc	144
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser	
35 40 45	
atc gtg cac agc aac ggc aac acc tac ctg gag tgg tac ctg cag aag	192
Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys	
50 55 60	
ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc	240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe	
65 70 75 80	
agc ggc gtg ccc gac cgg ttc agc ggc agc ggc agc ggc acc gac ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe	
85 90 95	
acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	
100 105 110	
tgc ttc cag ggc tcc cac gtg ccc tac acc ttc ggc cag ggc acc aag	384
Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys	
115 120 125	
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc	432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
130 135 140	
ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
145 150 155 160	
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
165 170 175	
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
180 185 190	
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
195 200 205	
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
210 215 220	
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
225 230 235	

<210> 99

<211> 239

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 99

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1 5 10 15
Gly Ala Tyr Gly Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro
20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45

Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
115 120 125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 100

<211> 48

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3L CDRL1

<220>

<221> CDS

<222> (1)..(48)

<400> 100

cgg agc agc cag agc atc gtg cac agc aac ggc aac acc tac ctg gag 48
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 101

<211> 16

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 101

Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 102

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<211> 21
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T3L CDRL2

<220>
<221> CDS
<222> (1)..(21)

<400> 102
aag gtg agc aac cgg ttc agc
Lys Val Ser Asn Arg Phe Ser
1             5

<210> 103
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 103
Lys Val Ser Asn Arg Phe Ser
1             5

<210> 104
<211> 24
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T3L CDRL3

<220>
<221> CDS
<222> (1)..(24)

<400> 104
ttc cag ggc tcc cac gtg ccc tac
Phe Gln Gly Ser His Val Pro Tyr
1             5

<210> 105
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 105
Phe Gln Gly Ser His Val Pro Tyr
1             5

<210> 106
<211> 1407
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T1H

<220>
<221> CDS

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21

24

<222> (1)..(1407)

<220>

<221> sig_peptide

<222> (1)..(57)

<400> 106

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atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg      48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1      5      10      15

gtg ctg agc cag gtg cag ctg gtg cag agc gcc gcc gag gtg aag aag      96
Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20     25     30

ccc gcc agc agc gtg aag gtg agc tgc aag gcc agc gcc tac acc ttc      144
Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35     40     45

acc cac tac agc atg cac tgg gtg cgg cag gcc ccc gcc cag gcc ctg      192
Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50     55     60

gag tgg atg gcc tgg atc aac acc tac acc gcc gag ccc acc tac gcc      240
Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65     70     75     80

gac gac ttc aag gcc cgg gtg acc atc acc gcc gac acc tcc acc tcc      288
Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser
85     90     95

acc gcc tac ctg gaa ctg agc agc ctg cgg agc gag gac acc gcc gtg      336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100    105    110

tac tac tgc gcc acc tac tac cgg tac gag cgg gac ttc gac tac tgg      384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
115    120    125

ggc cag gcc acc ctg gtg acc gtg agc tca gcc tcc acc aag gcc cca      432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130    135    140

agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct gcc gcc aca      480
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
145    150    155    160

gcc gcc ctg gcc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc      528
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165    170    175

gtg agc tgg aac tca gcc gcc ctg acc agc gcc gtg cac acc ttc ccc      576
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180    185    190

gct gtc ctg cag tcc tca gga etc tac tcc etc agc agc gtg gtg acc      624
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195    200    205

gtg ccc tcc agc agc ttg gcc acc cag acc tac atc tgc aac gtg aat      672
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210    215    220

cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct      720
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225    230    235    240

tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa etc ctg      768
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gln Leu Leu
245    250    255

ggg gga ccc tca gtc ttc etc ttc ccc cca aaa ccc aag gac acc etc      816
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260    265    270

atg atc tcc cgg acc cct gag gtc aca tgc ggg gtg gtg gac gtg agc      864
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275    280    285

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac gcc gtg gag      912
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290    295    300

```

```

gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg      960
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305                      310                      315                      320

tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat      1008
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325                      330                      335

ggc aag gag tac aag tgc aag gtc tcc aac aza gcc ctc cca gcc ccc      1056
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340                      345                      350

atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag      1104
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355                      360                      365

gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc      1152
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370                      375                      380

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg      1200
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385                      390                      395                      400

gag tgg gag agc aat gcc cag ccc gag aac aac tac aag acc acc cct      1248
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405                      410                      415

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc      1296
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420                      425                      430

gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg      1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435                      440                      445

atg cat gaq gct ctg cac aac cac tac acc caq aaq agc ctc tcc ctg      1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450                      455                      460

tct ccc ggc aaa tga
Ser Pro Gly Lys
465

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<210> 107

<211> 468

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 107

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Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1                      5                      10                      15

```

```

Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys

```

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

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 450 455 460

Ser Pro Gly Lys
 465

<210> 108

<211> 15

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T1H CDRH1

<220>

<221> CDS <222> (1)..(15)

<400> 108

cac tac agc atg cac
 His Tyr Ser Met His
 1 5

15

<210> 109

<211> 5

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 109

His Tyr Ser Met His
 1 5

<210> 110

<211> 51

<212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1H-CDRH2

<220>
 <221> CDS
 <222> (1)..(51)

<400> 110
 tgg atc aac acc tac acc ggc gag ccc acc tac gcc gac gac ttc aag 48
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15
 ggc 51
 Gly

<210> 111
 <211> 17
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 111
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15
 Gly

<210> 112
 <211> 30
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1H CDRH3

<220>
 <221> CDS
 <222> (1)..(30)

<400> 112
 tac tac cgg tac gag cgg gac ttc gac tac 30
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 113
 <211> 10
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 113
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 114
 <211> 1407
 <212> DNA
 <213> Artificial

<220>

<223> hSH348-1-T3H

<220>

<221> CDS

<222> (1)..(1407)

<220>

<221> sig_peptide

<222> (1)..(57)

<400> 114

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atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg      48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1          5          10         15

gtg ctg agc cag atc cag ctg gtg cag agc gcc gcc gag gtg aag aag      96
Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20         25         30

ccc gcc agc agc gtg aag gtg agc tgc aag gcc agc gcc tac acc ttc      144
Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35         40         45

acc cac tac agc atg cac tgg gtg cgg cag gcc ccc gcc cag gcc ctg      192
Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50         55         60

aag tgg atg gcc tgg atc aac acc tac acc gcc gag ccc acc tac gcc      240
Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65         70         75         80

gac gac ttc aag gcc cgg ttc gcc ttc agc ctg gac acc tcc acc tcc      288
Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
85         90         95

acc gcc tac ctg gaa ctg agc agc ctg cgg agc gag gac acc gcc gtg      336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100        105        110

tac tac tgc gcc acc tac tac cgg tac gag cgg gac ttc gac tac tgg      384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
115        120        125

ggc cag gcc acc ctg gtg acc gtg agc tca gcc tcc acc aag gcc cca      432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130        135        140

agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct gcc gcc aca      480
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
145        150        155        160

gcc gcc ctg gcc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc      528
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165        170        175

gtg agc tgg aac tca gcc gcc ctg acc agc gcc gtg cag acc ttc ccc      576
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180        185        190

gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc      624
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195        200        205

gtg ccc tcc agc agc ttg gcc acc cag acc tac atc tgc aac gtg aat      672
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210        215        220

cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct      720
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225        230        235        240

tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg      768
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245        250        255

ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc      816

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Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
260 265 270	
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc	864
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
275 280 285	
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag	912
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	
290 295 300	
gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg	960
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	
305 310 315 320	
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat	1008
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn	
325 330 335	
ggc aag gag tac aag tgc aag gtc tcc aac aza gcc ctc cca gcc ccc	1056
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro	
340 345 350	
atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag	1104
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln	
355 360 365	
gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc	1152
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val	
370 375 380	
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg	1200
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val	
385 390 395 400	
gag tgg gag agc aat gcc cag ccc gag aac aac tac aag acc acc cct	1248
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro	
405 410 415	
ccc gtg ctg gac tcc gac gcc tcc ttc ttc ctc tac agc aag ctc acc	1296
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr	
420 425 430	
gtg gac aag agc agg tgg cag cag gcc aac gtc ttc tca tgc tcc gtg	1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val	
435 440 445	
atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg	1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu	
450 455 460	
tct ccc gcc aaa tga	1407
Ser Pro Gly Lys	
465	

<210> 115

<211> 468

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 115

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

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

Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
85 90 95

Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

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

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
 225 230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 450 455 460

Ser Pro Gly Lys

465

<210> 116

<211> 15

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3H CDRH1

<220>

<221> CDS

<222> (1)..(15)

<400> 116

cac tac agc atg cac
 His Tyr Ser Met His
 1 5

15

<210> 117

<211> 5

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 117

His Tyr Ser Met His
1 5

<210> 118

<211> 51

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3H CDRH2

<220>

<221> CDS

<222> (1)..(51)

<400> 118

tgg atc aac acc tac acc ggc gag ccc acc tac gcc gac gac ttc aag 48
Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15
ggc 51
Gly

<210> 119

<211> 17

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 119

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15
Gly

<210> 120

<211> 30

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3H CDRH3

<220>

<221> CDS

<222> (1)..(30)

<400> 120

tac tac cgg tac gag cgg gac ttc gac tac 30
Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 121

<211> 10

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 121

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 122

<211> 720

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1L

<220>

<221> CDS

<222> (1)..(720)

<220>

<221> sig_peptide

<222> (1)..(60)

<400> 122

atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc	48
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser	
1 5 10 15	
ggc gca tat ggc gac ata gtg atg acc cag agc ccc ctg agc ctg ccc	96
Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro	
20 25 30	
gtg acc ccc ggc gag ccc gcc agc atc agc tgc cgg agc agc cag agc	144
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser	
35 40 45	
atc gtg cac agc agc ggc atc acc tac ctg gag tgg tac ctg cag aag	192
Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys	
50 55 60	
ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc	240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe	
65 70 75 80	
agc ggc gtg ccc gac cgg ttc agc ggc agc ggc agc ggc acc gac ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe	
85 90 95	
acc ctg aag atc agc cgg gtg gaa gcc gaa gac ctg ggc ctg tac tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	
100 105 110	
tgc ttc cag ggc agc cac gtg ccc tac acc ttc ggc cag ggc acc aag	384
Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys	
115 120 125	
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc	432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
130 135 140	
ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
145 150 155 160	
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
165 170 175	
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
180 185 190	
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
195 200 205	
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
210 215 220	
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
225 230 235	

<210> 123

<211> 239

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 123

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Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1          5          10          15

Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
20        25        30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35        40        45

Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
50        55        60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
65        70        75        80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85        90        95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100       105       110

Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
115       120       125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130       135       140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145       150       155       160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165       170       175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180       185       190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195       200       205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210       215       220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225       230       235

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<210> 124

<211> 48

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1L CDRL1

<220>

<221> CDS

<222> (1)..(48)

<400> 124

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cgg agc agc cag agc atc gtg cac agc agc ggc atc acc tac ctg gag      48
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
1          5          10          15

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<210> 125
 <211> 16
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 125
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 126
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L CDRL2

<220>
 <221> CDS
 <222> (1)..(21)

<400> 126
 aag gtg agc aac cgg ttc agc
 Lys Val Ser Asn Arg Phe Ser
 1 5 21

<210> 127
 <211> 7
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 127
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 128
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L-CDRL3

<220>
 <221> CDS
 <222> (1)..(24)

<400> 128
 ttc cag ggc agc cac gtg ccc tac
 Phe Gln Gly Ser His Val Pro Tyr
 1 5 24

<210> 129
 <211> 8
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 129

Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 130

<211> 720

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T3L

<220>

<221> CDS

<222> (1)..(720)

<220>

<221> sig_peptide

<222> (1)..(60)

<400> 130

atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc	48
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser	
1 5 10 15	
ggc gca tat ggc gac gtg ctg atg acc cag agc ccc ctg agc ctg ccc	96
Gly Ala Tyr Gly Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro	
20 25 30	
gtg acc ccc ggc gag ccc gcc agc atc agc tgc cgg agc agc cag agc	144
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser	
35 40 45	
atc gtg cac agc agc ggc atc acc tac ctg gag tgg tac ctg cag aag	192
Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys	
50 55 60	
ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc	240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe	
65 70 75 80	
agc ggc gtg ccc gac cgg ttc agc ggc agc ggc agc ggc acc gac ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe	
85 90 95	
acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	
100 105 110	
tgc ttc cag ggc agc cac gtg ccc tac acc ttc ggc cag ggc acc aag	384
Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys	
115 120 125	
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc	432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
130 135 140	
ccc tcc gac gag cag ctg aag tcc gcc acc gcc tcc gtg gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
145 150 155 160	
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
165 170 175	
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
180 185 190	
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
195 200 205	
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
210 215 220	
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
225 230 235	

<210> 131

<211> 239

<212> PRT

<213> Artificial <220>

<223> Synthetic Construct

<400> 131

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Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1          5          10          15

Gly Ala Tyr Gly Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro
20        25        30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35        40        45

Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
50        55        60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
65        70        75        80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85        90        95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100       105       110

Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
115       120       125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130       135       140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145       150       155       160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165       170       175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180       185       190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195       200       205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210       215       220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225       230       235

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<210> 132

<211> 48

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T3L CDRL1

<220>

<221> CDS

<222> (1)..(48)

<400> 132

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cgg agc agc cag agc atc gtg cac agc agc ggc atc acc tac ctg gag
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
1          5          10          15

```

48

<210> 133

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<211> 16
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 133
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
1          5          10          15

<210> 134
<211> 21
<212> DNA
<213> Artificial

<220>
<223> hSH357-1-T3L CDRL2

<220>
<221> CDS
<222> (1)..(21)

<400> 134
aag gtg agc aac cgg ttc agc
Lys Val Ser Asn Arg Phe Ser
1          5
21

<210> 135
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 135
Lys Val Ser Asn Arg Phe Ser
1          5

<210> 136
<211> 24
<212> DNA
<213> Artificial

<220>
<223> hSH357-1-T3L CDRL3

<220>
<221> CDS
<222> (1)..(24)

<400> 136
ttc cag ggc agc cac gtg ccc tac
Phe Gln Gly Ser His Val Pro Tyr
1          5
24

<210> 137
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 137

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Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 138

<211> 1407

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1H

<220>

<221> CDS

<222> (1)..(1407)

<220>

<221> sig_peptide

<222> (1)..(57)

<400> 138

atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg	48
Met Lys His Leu Trp Phe Phe Leu Leu Val Ala Ala Pro Arg Trp	
1 5 10 15	
gtg ctg agc cag gtg cag ctg gtg cag tcc gcc gcc gag gtg aag aag	96
Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys	
20 25 30	
ccc gcc gcc tcc gtg aag gtg tcc tgt aag gcc tcc gcc tac acc ttc	144
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe	
35 40 45	
atc gac tac tcc atg cac tgg gtg agg cag gcc ccc gcc cag gcc ctg	192
Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu	
50 55 60	
gag tgg atg gcc tgg atc aac acc tac acc gcc gag ccc acc tac tcc	240
Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser	
65 70 75 80	
gac gac ttc aag gcc agg gtg acc atc acc gcc gac acc tcc acc agc	288
Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser	
85 90 95	
acc gcc tac ctg gag ctg tcc tcc ctg agg tcc gag gac acc gcc gtg	336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val	
100 105 110	
tac tac tgt gcc acc tac tac agg tac gag agg gac ttc gac tac tgg	384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp	
115 120 125	
ggc cag gcc acc ctg gtg acg gtg agc tca gcc tcc acc aag gcc coa	432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct gcc gcc aca	480
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	
145 150 155 160	
gcc gcc ctg gcc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc	528
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr	
165 170 175	
gtg agc tgg aac tca gcc gcc ctg acc agc gcc gtg cac acc ttc ccc	576
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro	
180 185 190	

```

gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc 624
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat 672
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct 720
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225 230 235 240

tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg 768
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc 816
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc 864
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag 912
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg 960
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat 1008
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc 1056
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag 1104
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc 1152
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg 1200
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

gag tgg gag agc aat ggc cag ccc gag aac aac tac aag acc acc cct 1248
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc 1296
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg 1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg 1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

tct ccc ggc aaa tga 1407
Ser Pro Gly Lys
465

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<210> 139

<211> 468

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 139

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

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

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser
65 70 75 80

Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser
85 90 95

Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

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

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225 230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

Ser Pro Gly Lys
465

<210> 140

<211> 18

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1H CDRH1

<220>

<221> CDS

<222> (1)..(18)

<400> 140

atc gac tac tcc atg cac
Ile Asp Tyr Ser Met His
1 5

18

<210> 141

<211> 6

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 141

Ile Asp Tyr Ser Met His
1 5

<210> 142

<211> 51

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1H CDRH2

<220>

<221> CDS

<222> (1)..(51)

<400> 142

tgg atc aac acc tac acc ggc gag ccc acc tac tcc gac gac ttc aag
Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
1 5 10 15

48

ggc
Gly

51

<210> 143

<211> 17

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 143

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
1 5 10 15

Gly

<210> 144

<211> 30

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T1H CDRH3

<220>

<221> CDS

<222> (1)..(30)

<400> 144

tac tac agg tac gag aag gac ttc gac tac
Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

30

<210> 145

<211> 10

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 145

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 146

<211> 1407

<212> DNA

<213> Artificial

<220>

<223> hSH357-1-T3H

<220>

<221> CDS

<222> (1)..(1407)

<220>

<221> sig_peptide

<222> (1)..(57)

<400> 146

atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg	48
Met Lys His Leu Trp Phe Phe Leu Leu Val Ala Ala Pro Arg Trp	
1 5 10 15	
gtg ctg agc cag atc cag ctg gtg cag tcc gcc gcc gag gtg aag aag	96
Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys	
20 25 30	
ccc gcc gcc tcc gtg aag gtg tcc tgt aag gcc tcc gcc tac acc ttc	144
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe	
35 40 45	
atc gac tac tcc atg cac tgg gtg agg cag gcc ccc gcc cag gcc ctg	192
Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu	
50 55 60	
aag tgg atg gcc tgg atc aac acc tac acc gcc gag ccc acc tac tcc	240
Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser	
65 70 75 80	
gac gac ttc aag gcc agg ttc gcc ttc tcc ctg gac acc tcc acc agc	288
Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser	
85 90 95	
acc gcc tac ctg gag ctg tcc tcc ctg agg tcc gag gac acc gcc gtg	336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val	
100 105 110	
tac tac tgt gcc acc tac tac agg tac gag agg gac ttc gac tac tgg	384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp	
115 120 125	
ggc cag gcc acc ctg gtg acg gtg agc tca gcc tcc acc aag gcc cca	432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggc gcc aca	480

```

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

gcc gcc ctg gcc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc      528
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
          165          170          175

gtg agc tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccc      576
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
          180          185          190

gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc      624
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
          195          200          205

gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat      672
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
          210          215          220

cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct      720
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
          225          230          235          240

tgt gac aaa act ccc aca tgc cca ccc tgc cca gca cct gaa ctc ctg      768
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
          245          250          255

ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc      816
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
          260          265          270

atg atc tcc cgg acc cct gag gtc aca tgc g-g gtg gtg gac gtg agc      864
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
          275          280          285

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag      912
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
          290          295          300

gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc agc      960
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
          305          310          315          320

tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat      1008
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
          325          330          335

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc      1056
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
          340          345          350

atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag      1104
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
          355          360          365

gtg tac acc ctg ccc cca tcc cgg gag gag a-g acc aag aac cag gtc      1152
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
          370          375          380

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg      1200
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val

385          390          395          400

gag tgg gag agc aat ggc cag ccc gag aac acc tac aag acc acc cct      1248
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
          405          410          415

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc      1296
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
          420          425          430

gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg      1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
          435          440          445

atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg      1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
          450          455          460

tct ccc ggc aaa tga
Ser Pro Gly Lys
465

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<210> 147

<211> 468

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 147

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

355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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tgatgattaa acgtacgggt gccgccccct ccgtgttcac ctccccccc tccgacgagc      180
agctgagtc cggcacccgc tcctggtgtt gctgtctgaa taactttctac cccagagagg      240
ccaaggtgca gtggaagggt gacaacgccc tgcagtcagg gaactccag gagagcgtga      300
ccgagcagga cagcaaggac agcacctaca gctgagcag caccctgacc ctgagcaaaag      360
ccgactacga gaagcacaag gtgtacgcct gcgaggtgac ccaccagggc ctgagctccc      420
ccgtcaccaa gagcttcaac agggggggagt gttagggggc cgtttaaacg ggtggcatcc      480
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<210> 155

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gttagctcag cctccaccaa gggcccaagc gtcttcccc tggcacctc ctccaagagc      180
acctctggcg gcacagccgc cctgggctgc ctggtcaagg actacttccc cgaacccgtg      240
accgtgagct ggaactcagg cgcctcgacc agcggcgtgc acaccttccc cgtgtcctg      300
cagtcctcag gactctactc ctcagcagc gtggtgaccg tgcctccag cagcttgggc      360
accagacct acatctgcaa cgtgaatcac aagccagca acaccaagg ggacaagaga      420
gttgagocaa aatcttgtga caaaactcac acatgcccc cctgcccagc acctgaaact      480
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aatggcaagg agtacagtg caaggctctc aacaaagccc tcccagcccc catcgagaaa      780
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cgggaggaga tgaccaagaa ccaggctcag ctgacctgcc tggtaaaagg cttctatccc      900
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agcaggtggc agcagggcaa cgtctctcca tgcctcgtga tgcattgagg tctgcacaa      1080
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<210> 156

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<212> DNA

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caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg 360
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<210> 157

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<212> DNA

<213> Artificial

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<223> hSH348-1-T3L+signal

<400> 157

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gacccccggc gagcccgctt ccatcagctg ccggagcagc cagagcatcg tgcacagcaa 180
cggaacacac tacctggagt ggtacctgca gaagcccgcc cagagcccc agctgctgat 240
ctacaagggt agcaaccggt tcagcggcgt gcccgaccgg ttcagcggca gcggcagcgg 300
caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg 360
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<210> 158

<211> 460

<212> DNA

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<400> 158

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cagcagcgtg aagggtgagct gcaaggccag cggctacacc ttcacccact acagcatgca 180
ctggttgcgc cagccccccg gccagggcct ggaagtggatg ggtctgatac acacctacac 240
cgccgagccc acctacgccc acgaattcaa gggccgggtg accatcaccg ccgacacctc 300
cacctccacc gctacctgg aactgagcag cctgcggagc gaggacaccg ccgtgtacta 360
ctgcgccacc tactaccggt acgagcggga cttcgactac tggggccagc gcaaccctggt 420
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<210> 159

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cagcagcgtg aaggtgagct gcaaggccag cggctacacc ttcacccact acagcatgca 180
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cacctccacc gectacctgg aactgagcag cctgcggagc gaggacaccg ccgtgtacta 360
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gacccccggc gagcccgcca gcatcagctg ccggagcagc cagagcatcg tgcacagcag 180
cggcatcacc tacctggagt ggtacctgca gaagcccgcc cagagccccc agctgctgat 240
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caccgacttc accctgagga tcagccgggt ggaggccgag gacgtgggcg tgtactactg 360
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cggcatcacc tacctggagt ggtacctgca gaagcccgcc cagagccccc agctgctgat 240
ctacaagggt agcaaccggt tcagcggcgt gcccgaccgg ttcagcggca gcggcagcgg 300
caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg 360
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tacggtggcc gccccctccg tg 442

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<210> 162

<211> 460

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cgctccgtg aagggtgtct gtaaggcctc cggctacacc ttcctcgact actccatgca    180
ctgggtgagg caggcccccg gccagggcct ggagtggatg ggctggatca acacctacac    240
cggcgagccc acctactccg acgacttcaa gggcagggtg accatcaccc cgcacacctc    300
caccagcacc gctacctgg agctgtcttc cctgaggtcc gaggacaccg ccgtgtacta    360
ctgtgccacc tactacaggt acgagaggga cttcgactac tggggccagg gcaaccctggt    420
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cgctccgtg aagggtgtct gtaaggcctc cggctacacc ttcctcgact actccatgca    180
ctgggtgagg caggcccccg gccagggcct gaagtggatg ggctggatca acacctacac    240
cggcgagccc acctactccg acgacttcaa gggcaggttc gccttctccc tggacacctc    300
caccagcacc gctacctgg agctgtcttc cctgaggtcc gaggacaccg ccgtgtacta    360
ctgtgccacc tactacaggt acgagaggga cttcgactac tggggccagg gcaaccctggt    420
gacggtgagc tcagctctct ccaccaaggg cccctccgtg                    460

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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- [WO03094859A \[0006\]](#)
- [WO2006084226A \[0009\]](#)
- [WO9007861A \[0131\]](#)
- [US6972323B \[0131\]](#)
- [WO9708320A \[0138\]](#)
- [WO0105950A \[0138\]](#)
- [WO9201047A \[0140\]](#)
- [WO9220791A \[0140\]](#)
- [WO9306213A \[0140\]](#)
- [WO9311236A \[0140\]](#)
- [WO9319172A \[0140\]](#)
- [WO9501438A \[0140\]](#)
- [WO9515388A \[0140\]](#)
- [WO0231140A \[0144\]](#)
- [US4946778A \[0154\]](#)
- [US5260203A \[0154\]](#)

- [US5091513A \[0154\]](#)
- [US5455030A \[0154\]](#)
- [WO2004061104A \[0158\]](#)
- [US6214388B \[0177\]](#)
- [JP2007224007A \[0335\]](#)

Non-patent literature cited in the description

- Molecular and Cellular Biology, 1990, vol. 10, 6316-6324 [0002]
- Annual Review of Neuroscience, 1998, vol. 21, 309-345 [0003] [0033]
- Molecular Cancer Research, 2002, vol. 1, 79-87 [0003]
- Cancer Research, 2001, vol. 61, 2301-2306 [0004] [0005] [0005]
- International Journal of Cancer, 2003, vol. 103, 657-663 [0004] [0004]
- The Prostate, 1999, vol. 41, 275-280 [0004]
- American Journal of Pathology, 2003, vol. 163, 2271-2276 [0004]
- Cancer Science, 2005, vol. 96, 42-47 [0004]
- Clinical Cancer Research, 2003, vol. 9, 613-618 [0004] [0004]
- Oncology Reports, 2004, vol. 11, 605-611 [0004] [0004]
- Molecular Cancer Research, 2005, vol. 3, 541-551 [0004] [0005]
- Biochemical and Biophysical Research Communications, 2004, vol. 320, 1096-1102 [0005] [0005]
- Oncogene, 2004, vol. 23, 1448-1456 [0005] [0005]
- Cancer Research, 2002, vol. 62, 2840-2847 [0005] [0008]
- Oncogene, 2000, vol. 19, 6043-6052 [0006]
- Journal of Cell Science, 2004, vol. 117, 2037-2049 [0006]
- Oncogene, 2002, vol. 21, 7011-7026 [0006]
- The New England Journal of Medicine, 2007, vol. 357, 39-51 [0007]
- Oncogene, 2007, vol. 26, 3661-3678 [0007]
- Oncogene, 2007, vol. 26, 3603-3613 [0007]
- Nature Medicine, 2000, vol. 6, 443-446 [0007]
- Blood, 2004, vol. 103, 2738-2743 [0007]
- Cancer Research, 2003, vol. 63, 7907-7912 [0008]
- Neoplasia, 2006, vol. 8, 18-30 [0009]
- **SAIKI, R.K. et al.** Science, 1988, vol. 239, 487-489 [0052]
- **GLUZMAN, Y.** Cell, 1981, vol. 23, 175-182 [0055] [0143]
- **URLAUB, G. CHASIN, L.A.** Proc. Natl. Acad. Sci. USA, 1980, vol. 77, 4126-4220 [0055]
- **KOHLER MILSTEIN** Nature, 1975, vol. 256, 495-497 [0063]
- Monoclonal Antibodies Plenum Press 1980 0000 365-367 [0063]
- **WEIR, D.M.** Handbook of Experimental Immunology Blackwell Scientific Publications 1987 0000 vol. I. II. III, [0075]
- **KABAT, E.A. MAYER, M.M.** Experimental Immunochimistry Charles C Thomas Publisher 1964 0000 [0075] [0095]
- **KOHLER et al.** Nature, 1975, vol. 256, 495- [0089] [0099]
- **KOHLER et al.** Eur. J. Immunol., 1977, vol. 6, 511- [0089]
- **MILSTEIN et al.** Nature, 1977, vol. 266, 550- [0089] [0099]
- **WALSH** Nature, 1977, vol. 266, 495- [0089]
- **WEIR, D.M.** Handbook of Experimental Immunology Blackwell Scientific Publications 1987 0000 vol. I. II. III., [0095]
- **BARBARA, B.M. STANLEY, M.S.** Selected Methods in Cellular Immunology W.H. Freeman and Company 1980 0000 [0102]
- **WEIR, D.M.** Handbook of Experimental Immunology Blackwell Scientific Publications 1978 0000 vol. I, II, III, [0119]
- Proc. Natl. Acad. Sci. U.S.A., 1984, vol. 81, 6851-6855 [0130]
- Nature, 1986, vol. 321, 522-525 [0131]
- **TOMIZUKA, K. et al.** Nature Genetics, 1997, vol. 16, 133-143 [0132]
- **KUROIWA, Y. et al.** Nucl. Acids Res., 1998, vol. 26, 3447-3448 [0132]
- **YOSHIDA, H. et al.** Animal Cell Technology: Basic and Applied Aspects Kluwer Academic Publishers 1999 0000 vol. 10, 69-73 [0132]
- **TOMIZUKA, K. et al.** Proc. Natl. Acad. Sci. USA, 2000, vol. 97, 722-727 [0132]
- **WORMSTONE, I.M. et al.** Investigative Ophthalmology & Visual Science, 2002, vol. 43, 72301-2308 [0136]

- **CARMEN, S. et al.** Briefings in Functional Genomics and Proteomics, 2002, vol. 1, 2189-203 [0136]
- **SIRIWARDENA, D. et al.** Ophthalmology, 2002, vol. 109, 3427-431 [0136]
- Nature Biotechnology, 2005, vol. 23, 91105-1116 [0137] [0140]
- Annu. Rev. Immunol, 1994, vol. 12, 433-455 [0140]
- **URLAUB, G. CHASIN, L.A.** Proc. Natl. Acad. Sci. U.S.A., 1980, vol. 77, 4126-4220 [0143]
- **MILLSTEIN et al.** Nature, 1983, vol. 305, 537-539 [0152]
- **PLUCKTHUN** The Pharmacology of Monoclonal Antibodies Springer Verlag 1994 0000 vol. 113, 269-315 [0153]
- Nature Biotechnology, 2005, vol. 23, 1126-1136 [0153]
- **HUSTON, J.S. et al.** Proc. Natl. Acad. Sci. U.S.A., 1988, vol. 85, 5879-5883 [0154]
- Nature Biotechnology, 2005, vol. 23, 1137-1146 [0161]
- Strategies for Protein Purification and Characterization: A Laboratory Course Manual Cold Spring Harbor Laboratory Press 1996 0000 [0163]
- **ED HARLOW DAVID LANE** Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory 1988 0000 [0163]
- **SAMBROOK, J. FRITSCH, E.F. MANIATIS, T.** Molecular Cloning Cold Spring Harbor Laboratory Press 1989 0000 [0188]
- **EDMAN, P. et al.** Eur. J. Biochem., 1967, vol. 1, 80- [0241]
- **KABAT, E.A. et al.** Sequences of Proteins of Immunological Interest U.S. Department of Health and Human Services 1991 0000 vol. I and II, [0242]
- **HEINRICH, G. et al.** J. Exp. Med., 1984, vol. 159, 417-435 [0243]
- **KABAT, E.A. et al.** Sequence of Proteins of Immunological Interest U.S. Department of Health and Human Services 1991 0000 vol. I and II, [0246]
- Methods in Enzymology, 1991, vol. 203, 121-153 [0277]
- Nuc. Acid Res., 2007, vol. 35, D301-D303 [0277]
- **THORNTON et al.** J. Mol. Biol., 1996, vol. 263, 800-815 [0277]
- FEBS letters, 1996, vol. 399, 1-8 [0277]
- Proc. Natl. Acad. Sci. USA, 1989, vol. 86, 10029-10033 [0279]
- Nuc. Acid Res., 2001, vol. 29, 205-206 [0279]
- **QUEEN et al.** Proc. Natl. Acad. Sci. USA, 1989, vol. 86, 10029-10033 [0279]

ANTI-EPHA2-ANTISTOF

PATENTKRAV

1. Antistof, der specifikt binder til et EPHA2-polypeptid, der består af en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 426 til 534 ifølge SEQ ID NO: 8 i sekvenslisten, hvor
 5 antistoffet ikke har nogen evne til phosphorylering af EPHA2-tyrosinrester; og hvor de komplementaritetsbestemmende regioner er udvalgt fra følgende (i) til (iii):

(i) de komplementaritetsbestemmende regioner i den variable tungkædere region af antistoffet har aminosyresekvenserne ifølge enten SEQ ID NO: 59, 61 og 63 eller SEQ ID NO: 71, 73 og 75, og de komplementaritetsbestemmende regioner i den variable letkædere region af antistoffet har
 10 aminosyresekvenserne ifølge enten SEQ ID NO: 65, 67 og 69 eller SEQ ID NO: 77, 79 og 81;

(ii) antistoffet har aminosyresekvensen ifølge SEQ ID NO: 109 som CDRH₁, 111 som CDRH₂ og 113 som CDRH_{3r} og har aminosyresekvensen ifølge SEQ ID NO: 93 som CDRL₁, 95 som CDRL₂ og 97 som CDRL₃; eller

(iii) antistoffet har aminosyresekvensen ifølge SEQ ID NO: 141 som CDRH₁, 143 som CDRH₂ og
 15 145 som CDRH₃ og har aminosyresekvensen ifølge SEQ ID NO: 125 som CDRL₁, 127 som CDRL₂ og 129 som CDRL₃.

2. Antistof ifølge krav 1, hvor antistoffet kan opnås ved humanisering af et antistof, der består af følgende 1) og 2):

1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved
 20 aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 35 i sekvenslisten, eller et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 39 i sekvenslisten; og

2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 37 i sekvenslisten, eller et letkædepolypeptid, der
 25 omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 41 i sekvenslisten.

3. Antistof ifølge krav 2, hvor antistoffet kan opnås ved humanisering af et antistof, der består af følgende 1) og 2):

1) et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO:
 30 35 i sekvenslisten, eller et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 39 i sekvenslisten; og

2) et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 37 i sekvenslisten eller et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 41 i sekvenslisten.

35 4. Antistof ifølge krav 1, hvor antistoffet udviser en effekt med reduktion af et EPHA2-proteinniveau.

5. Antistof ifølge krav 1, hvor antistoffet ikke udviser en effekt med reduktion af et EPHA2-proteinniveau.

-2-

6. Antistof ifølge et hvilket som helst af kravene 1 til 5, hvor antistoffet specifikt binder til et peptid, der består af en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 439 til 534 ifølge SEQ ID NO: 8 i sekvenslisten.
7. Antistof ifølge et hvilket som helst af kravene 1 til 6, hvor antistoffet hæmmer phosphoryleringen af EPHA2-tyrosinrester, der er induceret af en EPHA2-ligand.
8. Antistof ifølge et hvilket som helst af kravene 1 til 6, hvor antistoffet ikke hæmmer EPHA2-ligandbinding til EPHA2, men hæmmer phosphoryleringen af EPHA2-tyrosinrester, der er induceret af liganden.
9. Antistof ifølge krav 1, hvor antistoffet har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 59, 61, og 63 i sekvenslisten som de komplementaritetsbestemmende regioner i den variable tungkædereion og har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 65, 67, og 69 i sekvenslisten, som komplementaritetsbestemmende områder i den variable letkædereion.
10. Antistof ifølge krav 9, hvor antistoffet har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 109 som CDRH₁, 111 som CDRH₂ og 113 som CDRH₃ og har aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 93 som CDRL₁, 95 som CDRL₂ og 97 som CDRL₃ i sekvenslisten.
11. Antistof ifølge krav 1 kendetegnet ved følgende 1) og 2):

1) med et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved den almene formel (I):



- 20 hvor FRH₁ repræsenterer en vilkårlig aminosyresekvens, der består af 18 til 30 aminosyrer; CDRH₁ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 59 i sekvenslisten; FRH₂ repræsenterer en vilkårlig aminosyresekvens, der består af 14 aminosyrer; CDRH₂ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 61 i sekvenslisten; FRH₃ repræsenterer en vilkårlig aminosyresekvens, der består af 32 aminosyrer; CDRH₃ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 63 i sekvenslisten; og FRH₄ repræsenterer en vilkårlig aminosyresekvens, der består af 11 aminosyrer, hvor disse aminosyrer er bundet til hinanden via peptidbindinger; og

2) med et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved den almene formel (II):

- 30 $-FRL_1-CDRL_1-FRL_2-CDRL_2-FRL_3-CDRL_3-FRL_4-(II)$

- hvor FRL₁ repræsenterer en vilkårlig aminosyresekvens, der består af 23 aminosyrer; CDRL₁ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 65 i sekvenslisten; FRL₂ repræsenterer en vilkårlig aminosyresekvens, der består af 15 aminosyrer; CDRL₂ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 67 i sekvenslisten; FRL₃ repræsenterer en vilkårlig aminosyresekvens, der består af 32 aminosyrer; CDRL₃ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 69 i sekvenslisten; og FRL₄ repræsenterer en vilkårlig aminosyresekvens, der består af 10 aminosyrer, hvor disse aminosyrer er bundet til hinanden via peptidbindinger.

12. Antistof ifølge krav 1, hvor antistoffet har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 71, 73, og 75 i sekvenslisten som komplementaritetsbestemmende områder i den variable tungkædere region og har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 77, 79 og 81 i sekvenslisten som komplementaritetsbestemmende områder i den variable letkædere region.

5 13. Antistof ifølge krav 12, hvor antistoffet har aminosyresekvenserne, der er repræsenteret ved SEQ ID NO: 141 som CDRH₁, 143 som CDRH₂ og 145 som CDRH₃ og har aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 125 som CDRL₄, 127 som CDRL₂ og 129 som CDRL₃ i sekvenslisten.

14. Antistof ifølge krav 1, der er kendetegnet ved følgende 1) og 2):

1) det har et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved den almene formel (I):



hvor FRH₁ repræsenterer en vilkårlig aminosyresekvens, der består af 18 til 30 aminosyrer; CDRH₁ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 71 i sekvenslisten; FRH₂ repræsenterer en vilkårlig aminosyresekvens, der består af 14 aminosyrer; CDRH₂ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 73 i sekvenslisten; FRH₃ repræsenterer en vilkårlig aminosyresekvens, der består af 32 aminosyrer; CDRH₃ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 75 i sekvenslisten; og FRH₄ repræsenterer en vilkårlig aminosyresekvens, der består af 11 aminosyrer, hvor disse aminosyrer er bundet til hinanden via peptidbindinger; og

2) det har et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved den almene formel (II):



hvor FRL₁ repræsenterer en vilkårlig aminosyresekvens, der består af 23 aminosyrer; CDRL₁ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 77 i sekvenslisten; FRL₂ repræsenterer en vilkårlig aminosyresekvens, der består af 15 aminosyrer; CDRL₂ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 79 i sekvenslisten; FRL₃ repræsenterer en vilkårlig aminosyresekvens, der består af 32 aminosyrer; CDRL₃ repræsenterer aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 81 i sekvenslisten; og FRL₄ repræsenterer en vilkårlig aminosyresekvens, der består af 10 aminosyrer, hvor disse aminosyrer er bundet til hinanden via peptidbindinger.

15. Antistof ifølge et hvilket som helst af kravene 1, 4 til 14, kendetegnet ved, at antistoffet er et humaniseret antistof.

16. Antistof ifølge et hvilket som helst af kravene 1 til 14, kendetegnet ved, at antistoffet er et humant antistof.

17. Antistof ifølge et hvilket som helst af kravene 1 til 14, kendetegnet ved, at antistoffet er et IgG-antistof.

18. Antistof ifølge et hvilket som helst af kravene 1 til 8, kendetegnet ved, at antistoffet er et hvilket som helst, der er udvalgt fra Fab, F(ab')₂, Fv, scFv, et diabody, et lineært antistof og et multispecifikt antistof.

19. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):
- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 35 i sekvenslisten, eller et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 39 i sekvenslisten; og
 - 2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 37 i sekvenslisten, eller et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 41 i sekvenslisten.
20. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):
- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 35 i sekvenslisten, og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 37 i sekvenslisten; og
 - 2) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 119 ifølge SEQ ID NO: 39 i sekvenslisten, og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 1 til 112 ifølge SEQ ID NO: 41 i sekvenslisten.
21. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):
- 1) et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 35 i sekvenslisten, eller et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 39 i sekvenslisten; og
 - 2) et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 37 i sekvenslisten eller et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 41 i sekvenslisten.
22. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):
- 1) et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 35 i sekvenslisten, og et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 37 i sekvenslisten; og
 - 2) et tungkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 39 i sekvenslisten og et letkædepolypeptid, der omfatter aminosyresekvensen, der er repræsenteret ved SEQ ID NO: 41 i sekvenslisten.
23. Antistof, der er produceret hybridom SH348-1 (FERM BP-10836).
24. Antistof, der er produceret hybridom SH357-1 (FERM BP-10837).
25. Antistof, der er opnået ved humanisering af et antistof ifølge et hvilket som helst af kravene 20, 22, 23 eller 24, hvor antistoffet specifikt binder til et EPHA2-polypeptid, der består af en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 426 til 534 ifølge SEQ ID NO: 8 i sekvenslisten, og hvor antistoffet ikke har evnen til phosphorylering af EPHA2-tyrosinrester.
26. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):

- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 107 i sekvenslisten, eller et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 115 i sekvenslisten; og
- 5 2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 91 i sekvenslisten eller et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 99 i sekvenslisten.
27. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):
- 10 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 107 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 91 i sekvenslisten; og
- 2) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 115 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 99 i sekvenslisten.
- 15 28. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):
- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 139 i sekvenslisten, eller et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 147 i sekvenslisten; og
- 20 2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 123 i sekvenslisten eller et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 131 i sekvenslisten.
- 25 29. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):
- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 139 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 123 i sekvenslisten; og
- 30 2) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 468 ifølge SEQ ID NO: 147 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 239 ifølge SEQ ID NO: 131 i sekvenslisten.
- 35 30. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):
- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 107 i sekvenslisten, eller et tungkædepolypeptid, der

omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 115 i sekvenslisten; og

- 2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 91 i sekvenslisten, eller et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 99 i sekvenslisten.

31. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):

- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 107 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 91 i sekvenslisten; og

- 2) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 115 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 99 i sekvenslisten.

32. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) og 2):

- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 139 i sekvenslisten, eller et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 147 i sekvenslisten; og

- 2) et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 123 i sekvenslisten eller et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 131 i sekvenslisten.

33. Antistof ifølge krav 1, hvor antistoffet består af følgende 1) eller 2):

- 1) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 139 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 123 i sekvenslisten; og

- 2) et tungkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 20 til 138 ifølge SEQ ID NO: 147 i sekvenslisten og et letkædepolypeptid, der omfatter en aminosyresekvens, der er repræsenteret ved aminosyrenumrene 21 til 134 ifølge SEQ ID NO: 131 i sekvenslisten.

34. Fab, F(ab')₂, Fv, scFv, et diabody, et lineært antistof eller et multispecifikt antistof, der kan afledes af et hvilket som helst antistof ifølge krav 21, 22 eller 25 til 29.

35. Musehybridom SH348-1 (FERM BP-10836).

36. Musehybridom SH357-1 (FERM BP-10837).

37. Farmaceutisk sammensætning kendetegnet ved, at den omfatter mindst ét antistof, der er udvalgt fra antistofferne ifølge krav 1 til 34.

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38. Farmaceutisk sammensætning til cancerbehandling kendetegnet ved, at den omfatter mindst ét antistof, der er udvalgt fra antistofferne ifølge krav 1 til 34.
39. Antistof eller farmaceutisk sammensætning ifølge krav 1 til 34, 37 eller 38 til anvendelse i en fremgangsmåde til hæmning af tumorvækst hos et pattedyr.
- 5 40. Anvendelse af et antistof eller en farmaceutisk sammensætning ifølge krav 1 til 34, 37 eller 38 til fremstillingen af et medikament til hæmning af tumorvækst hos et pattedyr.
41. Antistof eller farmaceutisk sammensætning ifølge krav 39 eller anvendelse eller farmaceutisk sammensætning ifølge krav 40, kendetegnet ved, at tumoren er en tumor, der udtrykker EPHA2.
42. Polynukleotid, der koder for et antistof eller et polypeptid ifølge et hvilket som helst af kravene 1
10 til 34.
43. Værtscelle, der er transformeret med et polynukleotid ifølge krav 42.
44. Fremgangsmåde til fremstilling af et antistof ved anvendelse af en værtscelle ifølge krav 43.

DRAWINGS

Figure 1

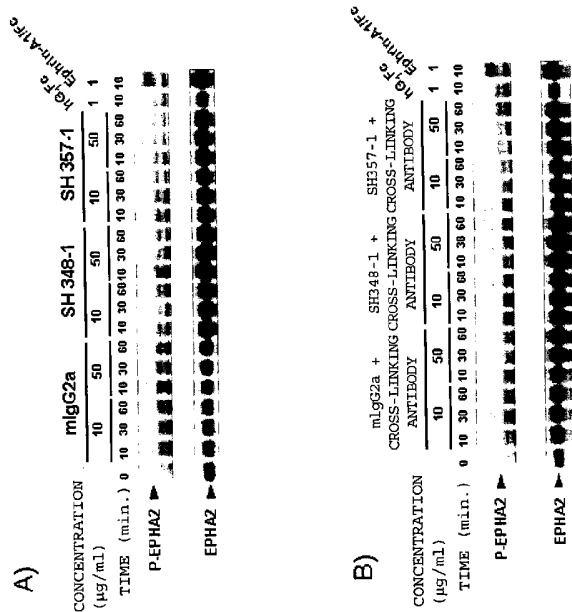


Figure 2

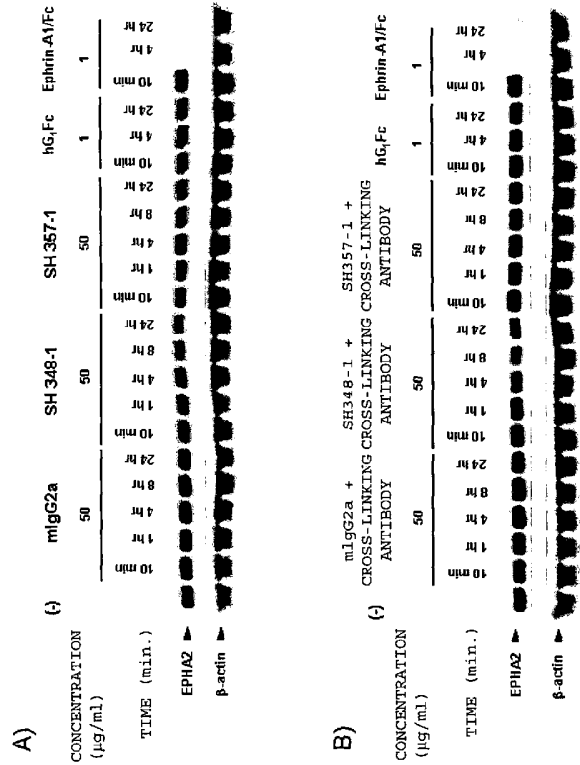


Figure 3

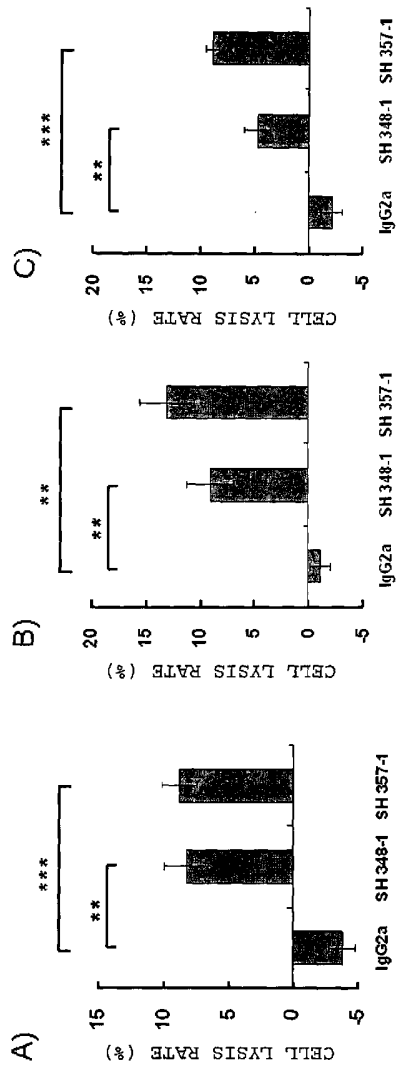


Figure 4

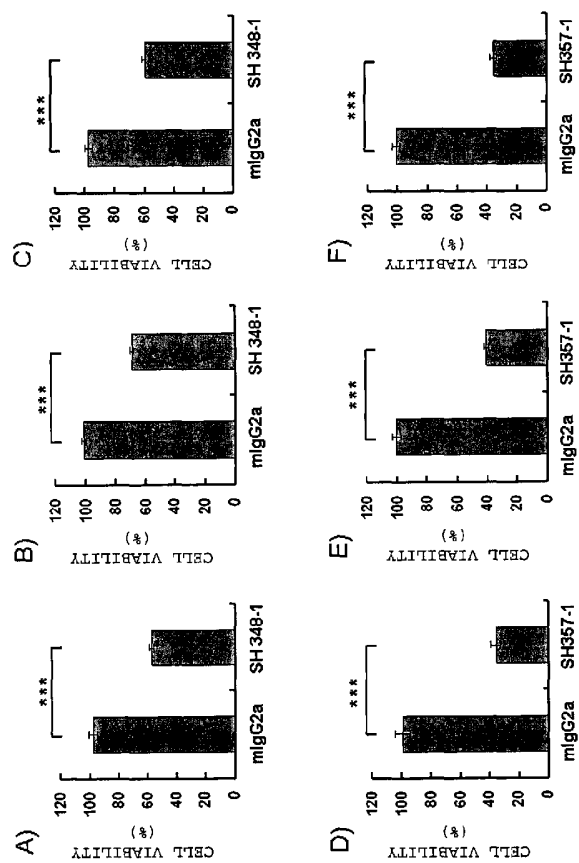


Figure 5

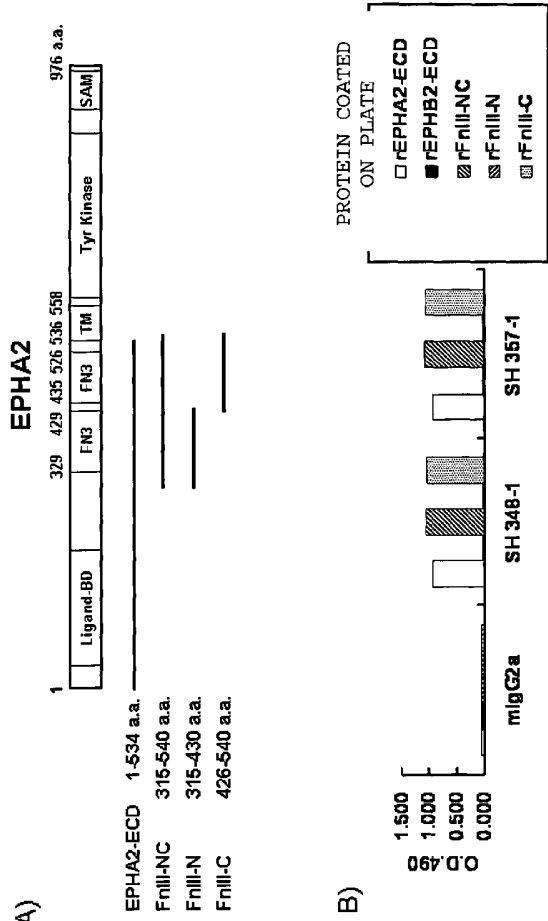


Figure 6

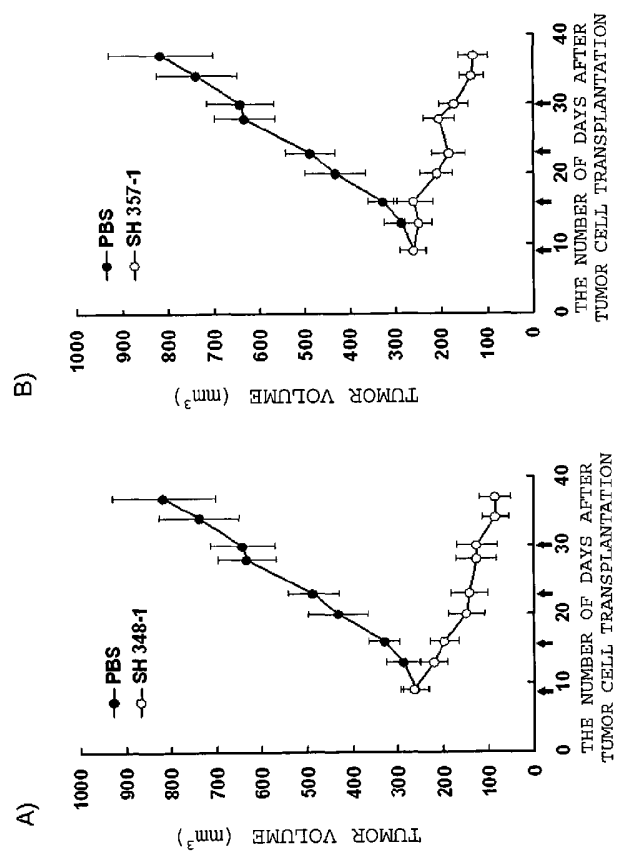


Figure 7

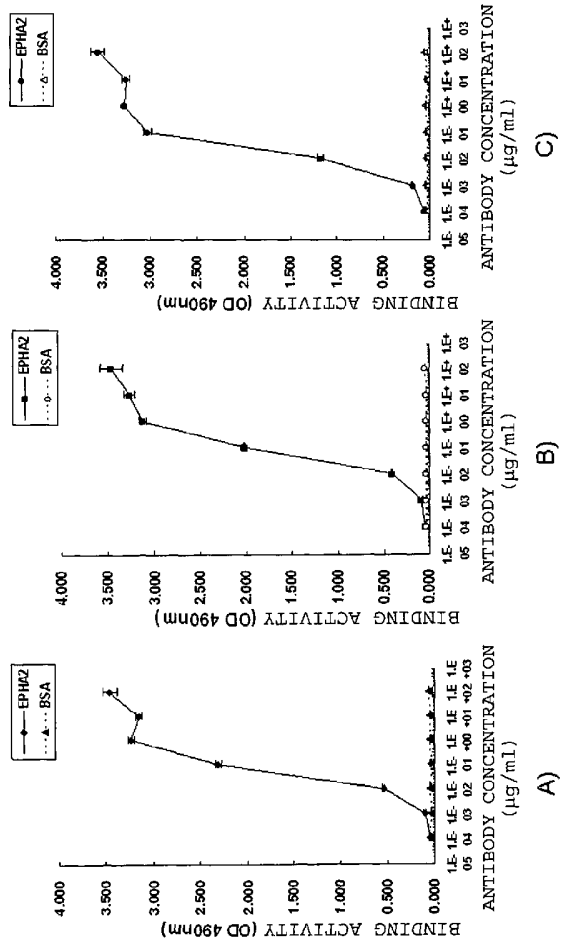


Figure 8

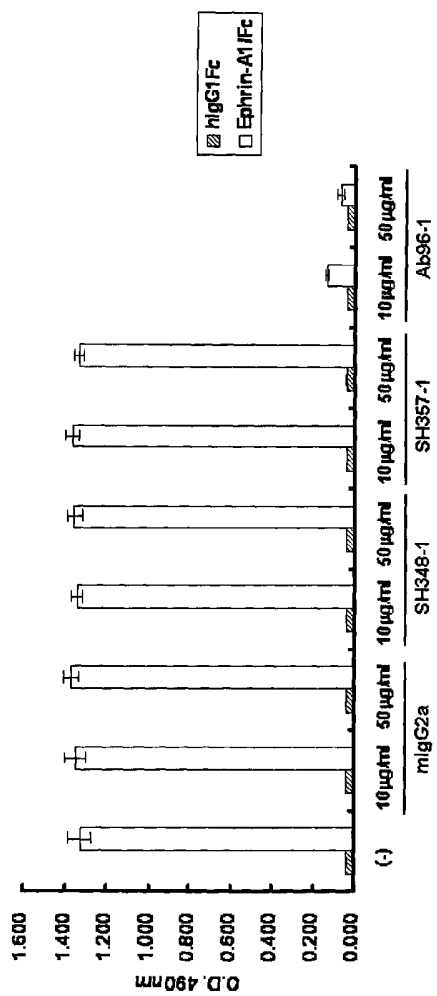


Figure 9

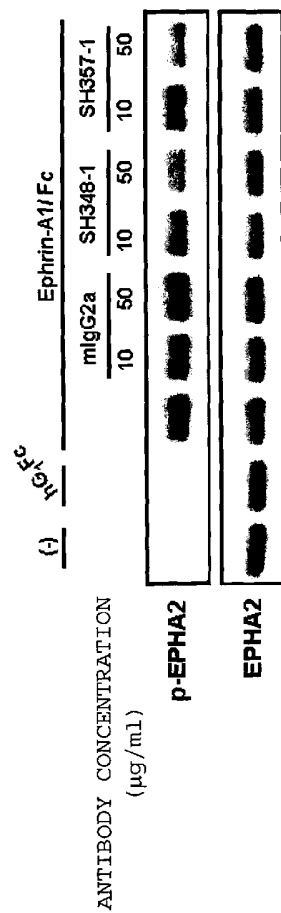


Figure 10

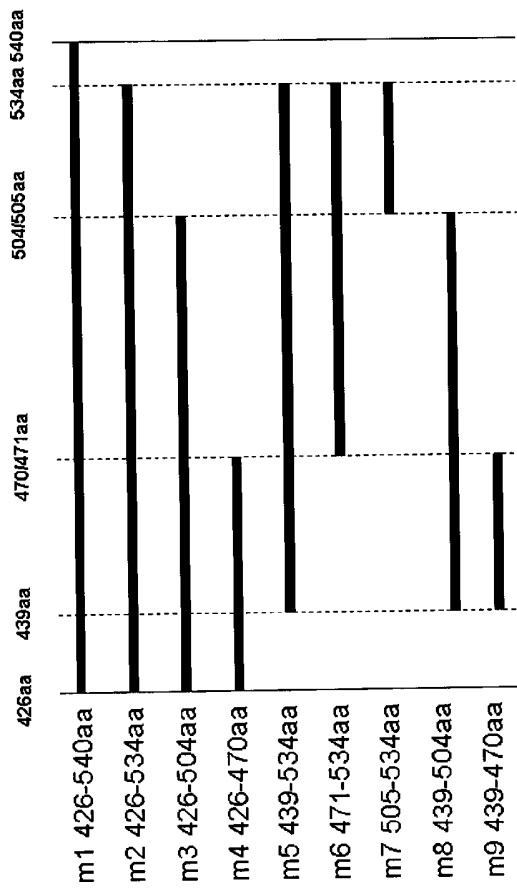


Figure 11

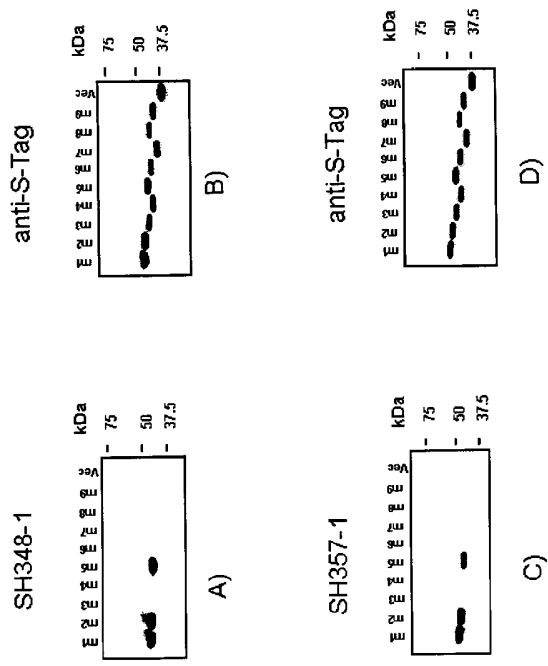


Figure 12

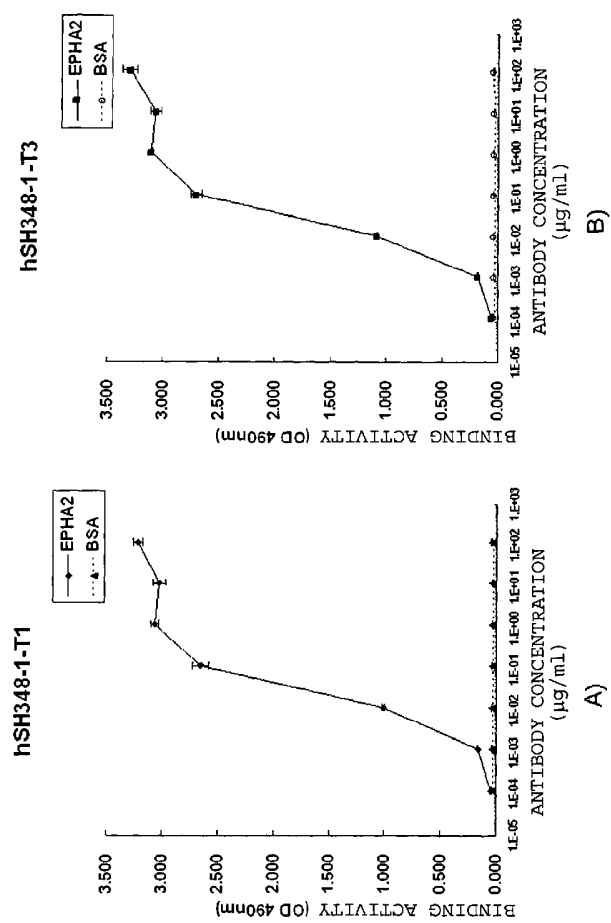


Figure 12

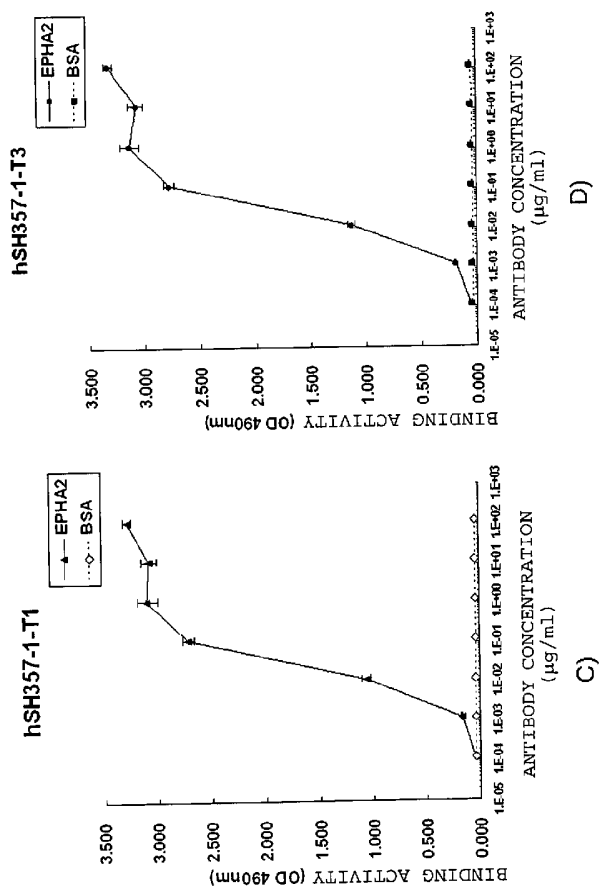


Figure 13

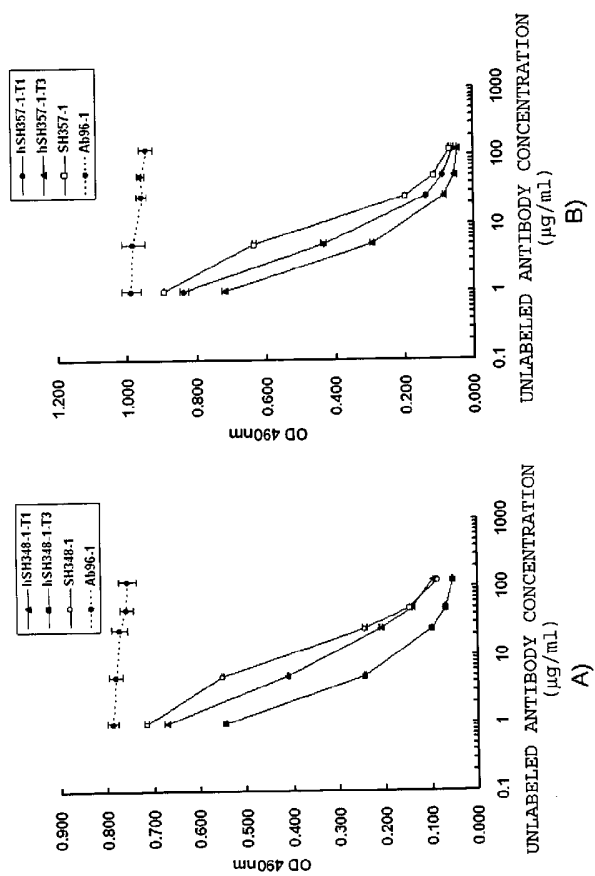


Figure 14

