



US 20040258684A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0258684 A1**

Fukushima et al.

(43) **Pub. Date: Dec. 23, 2004**

(54) **AGONIST ANTIBODIES**

(30) **Foreign Application Priority Data**

(75) Inventors: **Naoshi Fukushima**, Gotemba-shi (JP);
Masayuki Tsuchiya, Gotemba-shi (JP);
Masayoshi Oh-eda, Gotemba-shi (JP);
Shinsuke Uno, Gotemba-shi (JP);
Yasufumi Kikuchi, Gotemba-shi (JP);
Toshihiko Ohtomo, Gotemba-shi (JP)

Apr. 17, 2000 (JP) 2000-115246
Oct. 20, 2000 (JP) 2000-321821
Oct. 20, 2000 (JP) 2000-321822
Mar. 12, 2001 (WO) PCT/JP01/01912

Publication Classification

(51) **Int. Cl.⁷** **G01N 33/53**; A61K 39/395;
C07K 16/44

(52) **U.S. Cl.** **424/132.1**; 530/387.3; 435/7.1

Correspondence Address:
FOLEY AND LARDNER
SUITE 500
3000 K STREET NW
WASHINGTON, DC 20007 (US)

(57) **ABSTRACT**

(73) Assignee: **Chugai Seiyaku Kabushiki Kaisha**

Modified antibodies containing 2 or more H chain V domains and or more L chain V domains of a monoclonal antibody which can transduce a signal into cells by crosslinking a cell surface molecule, thereby serving as an agonist. Because of being usable as agonists for signal transduction, these modified antibodies are useful as, for example, preventives and/or remedies for various diseases such as cancer, inflammation, hormone disorders and blood diseases.

(21) Appl. No.: **10/645,085**

(22) Filed: **Oct. 7, 2002**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/JP01/03288, filed on Apr. 17, 2001.

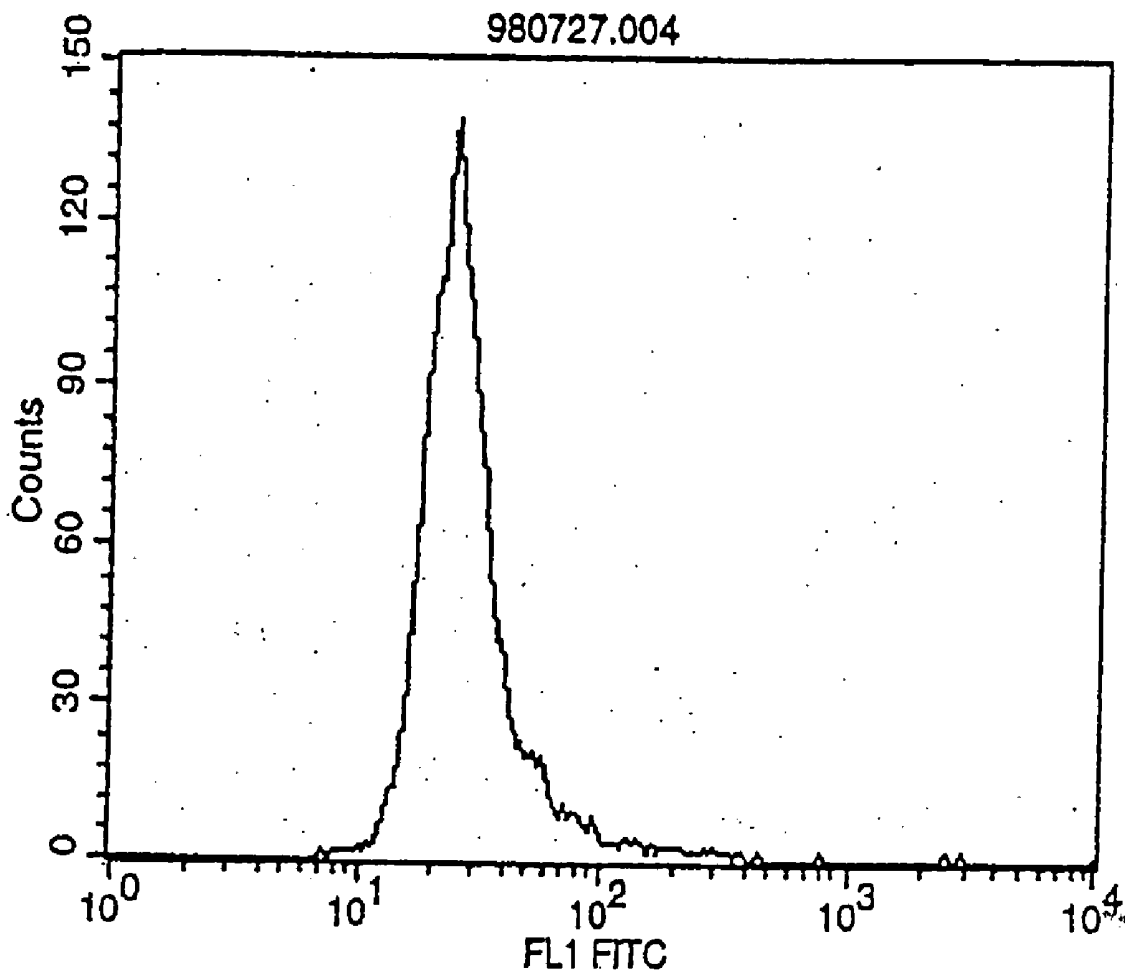


Fig. 1

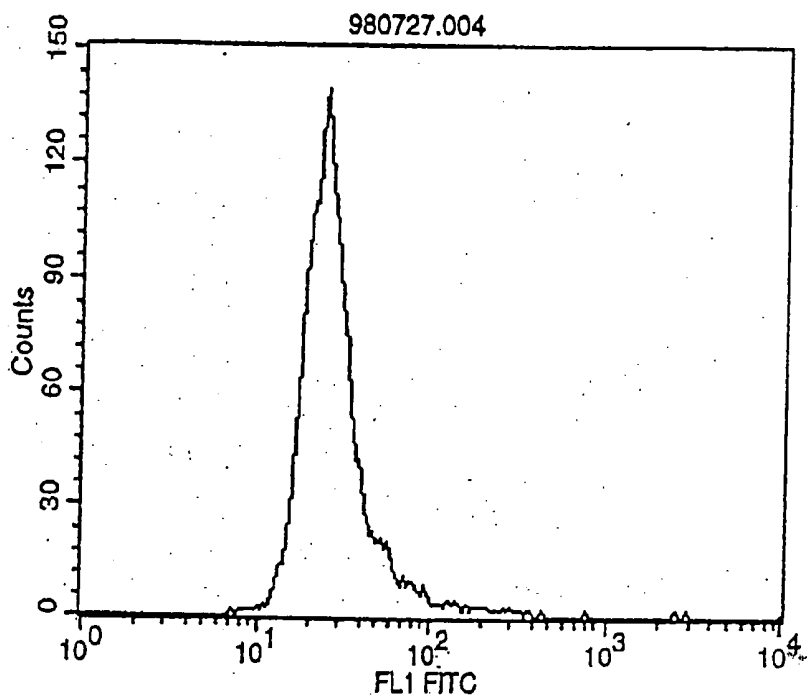


Fig. 2

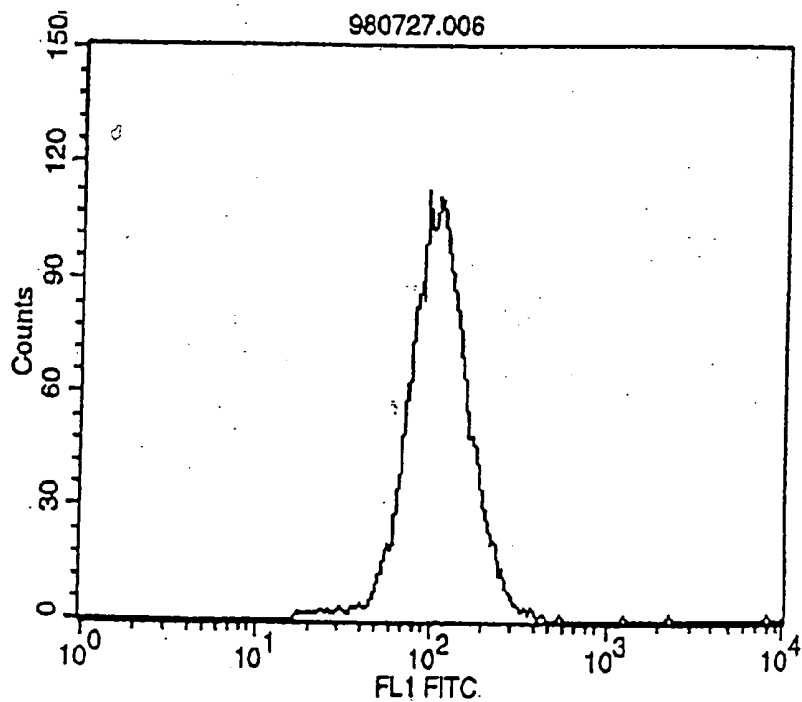


Fig. 3

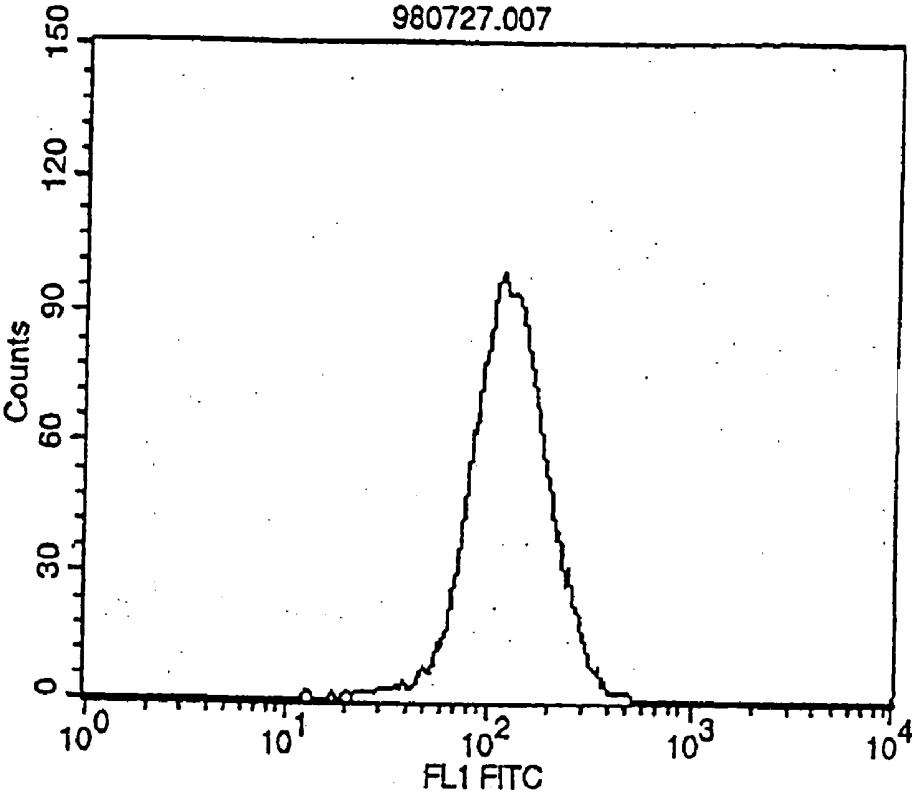


Fig. 4

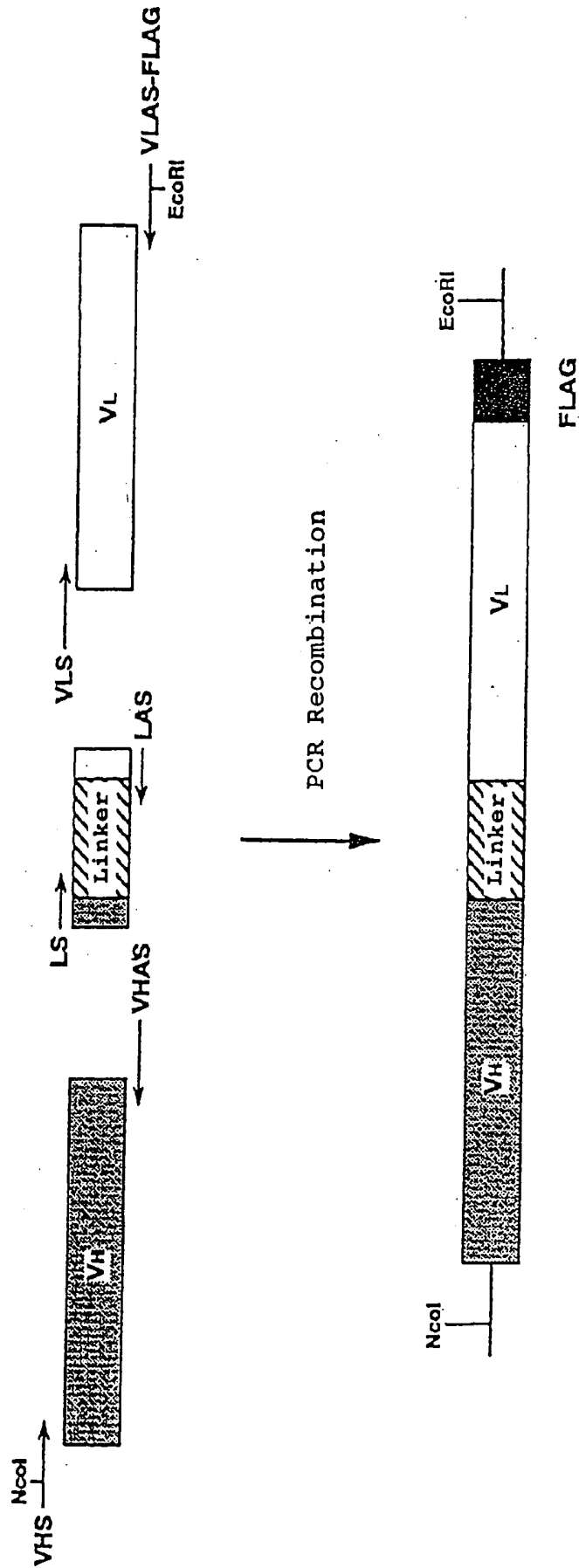


Fig. 5

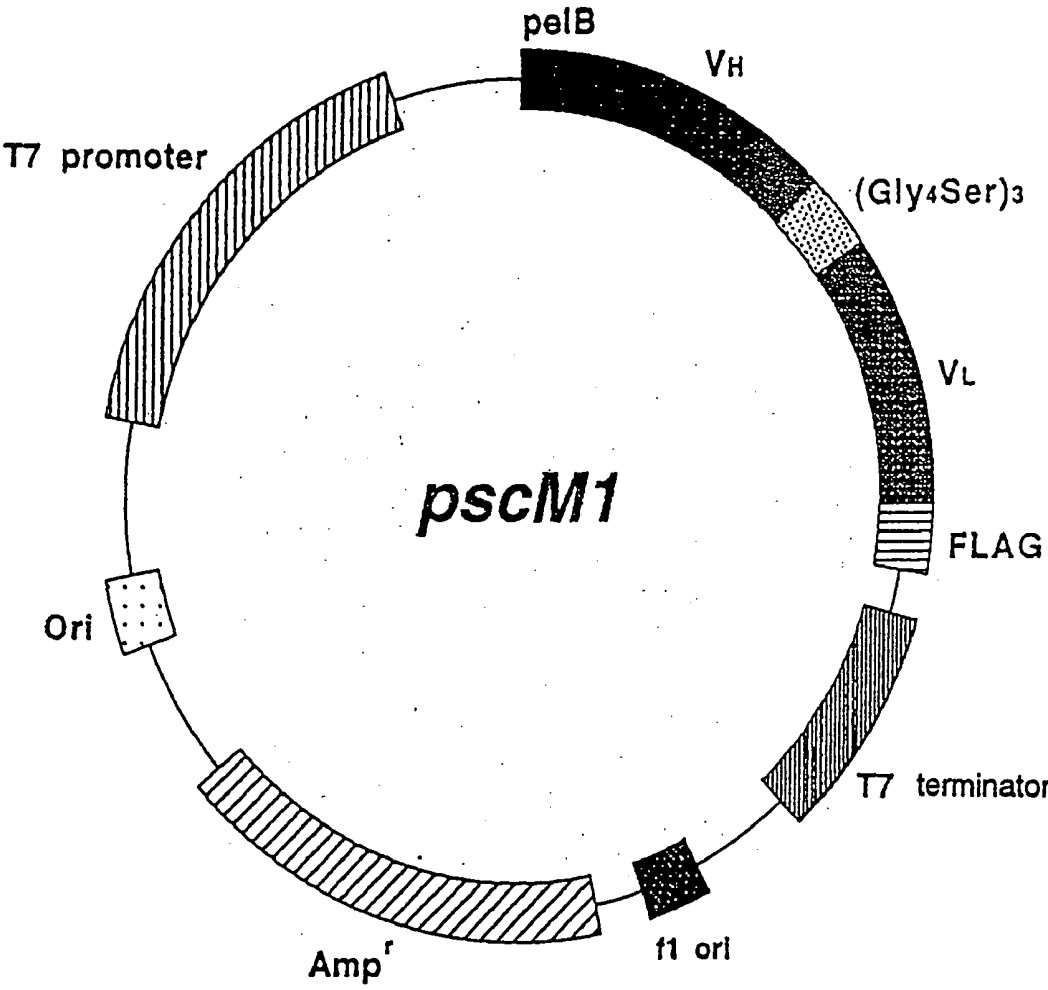


Fig. 6

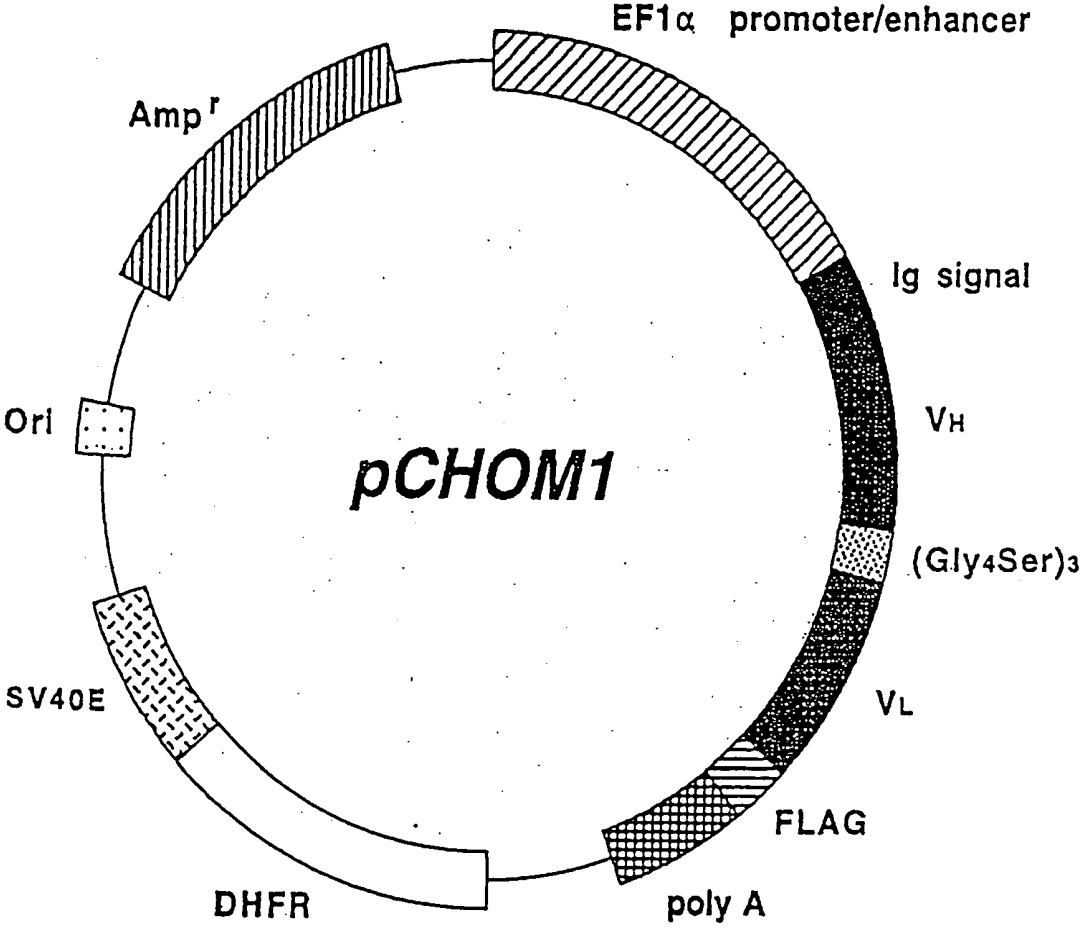


Fig. 7

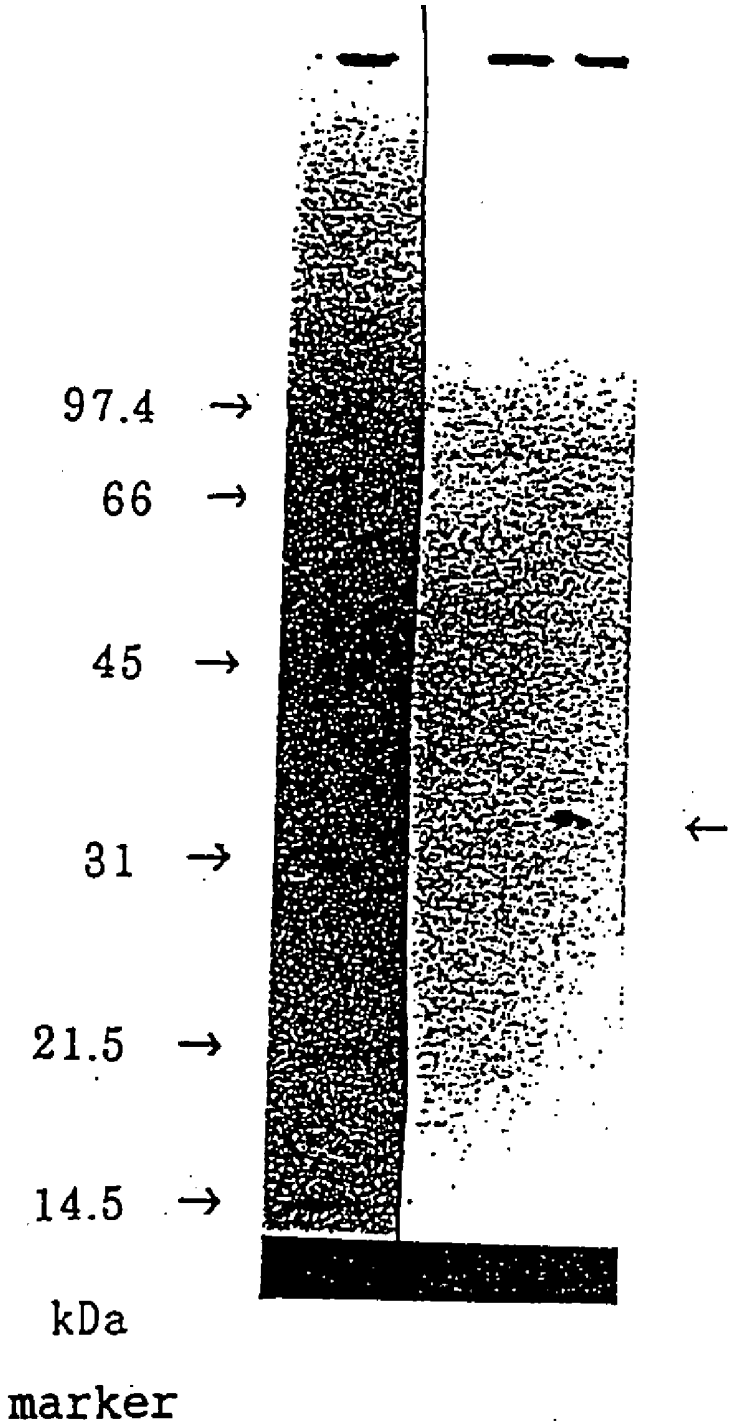


Fig. 8

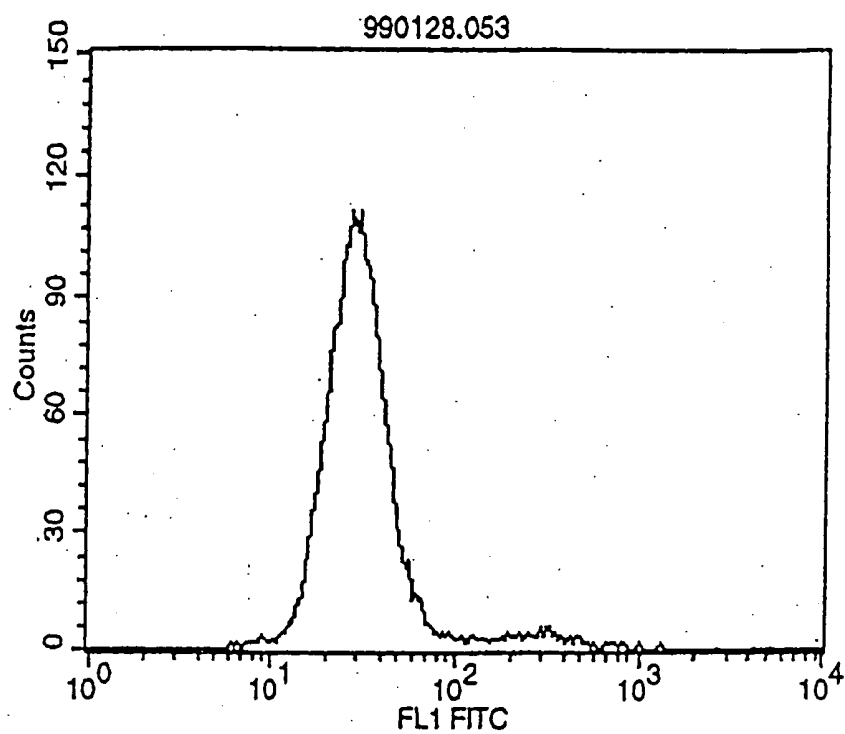


Fig. 9

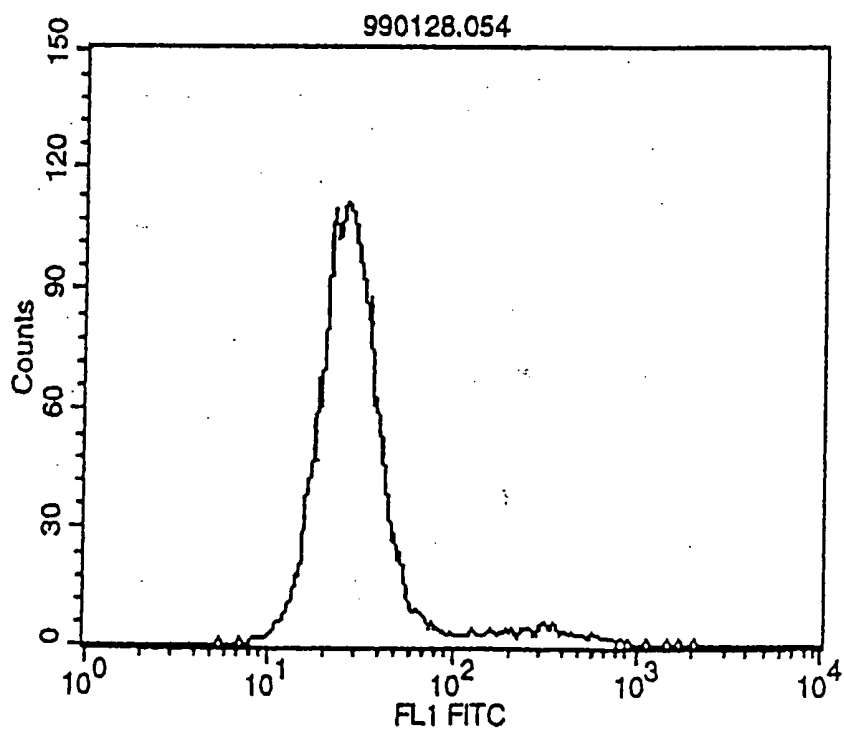


Fig. 10

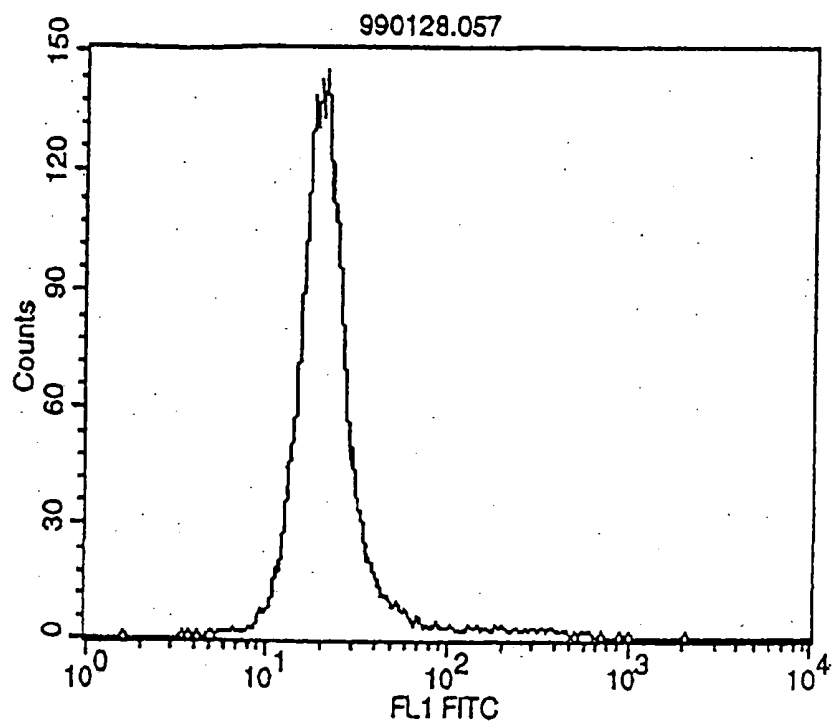


Fig. 11

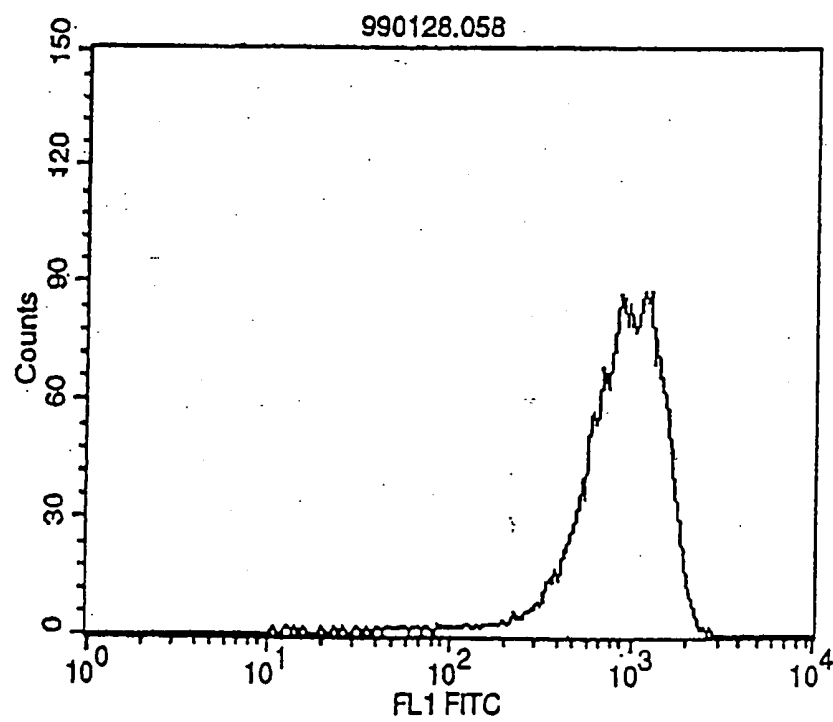


Fig. 12

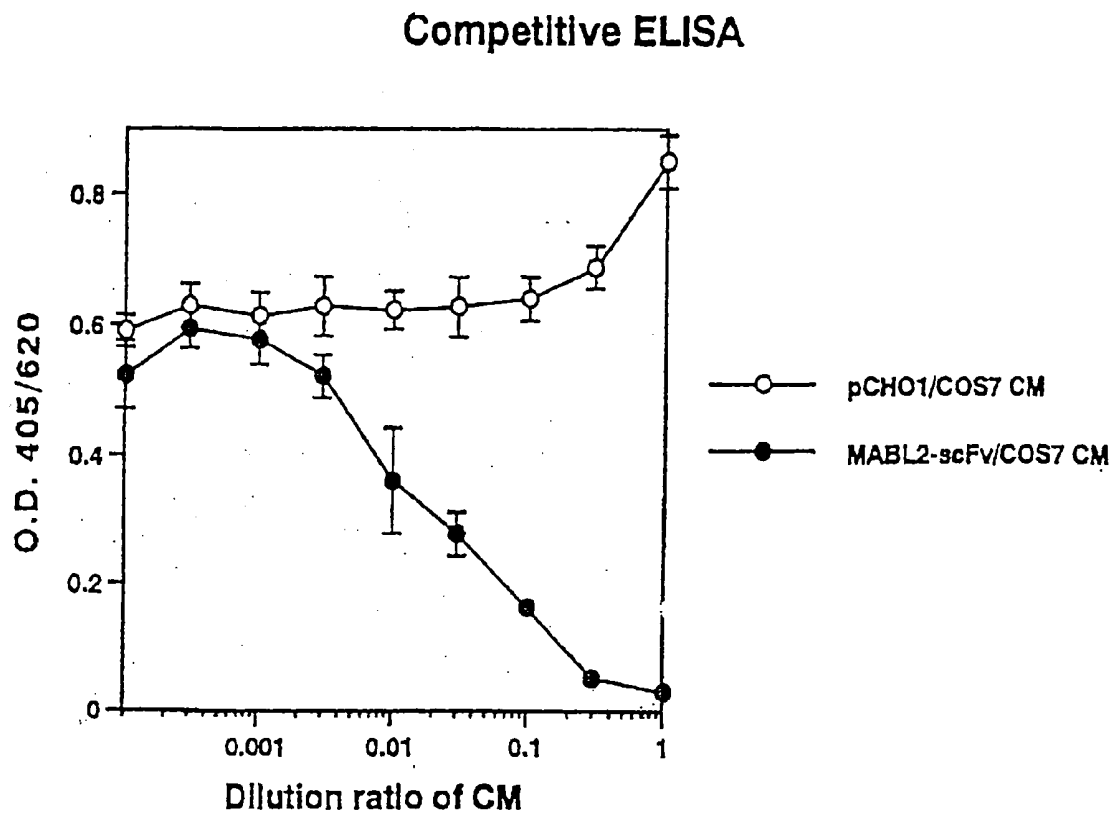


Fig. 13

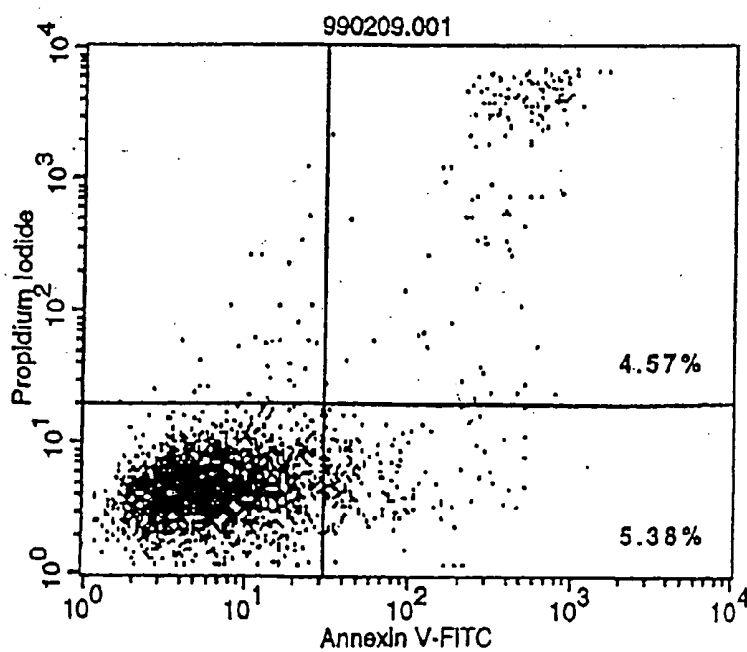


Fig. 14

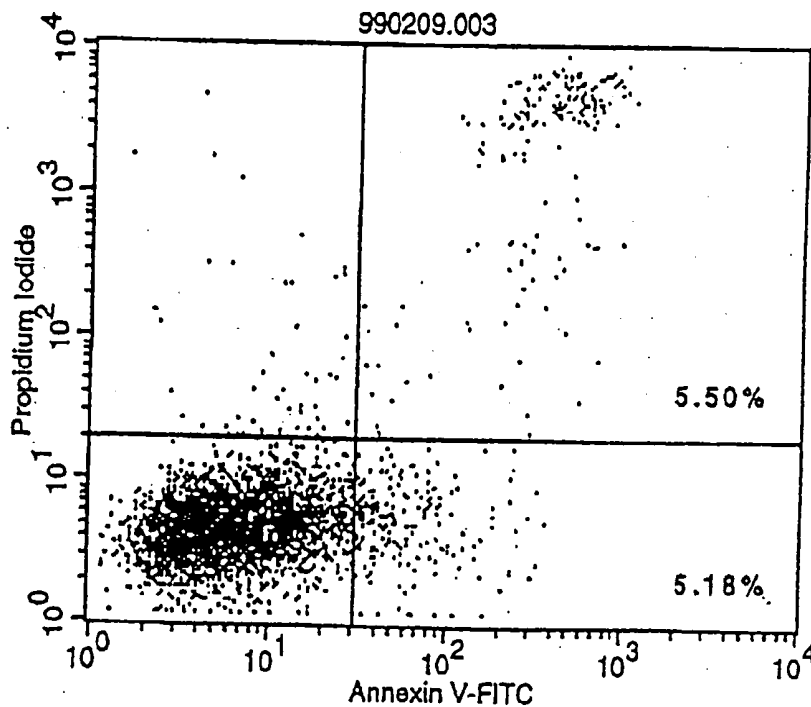


Fig. 15

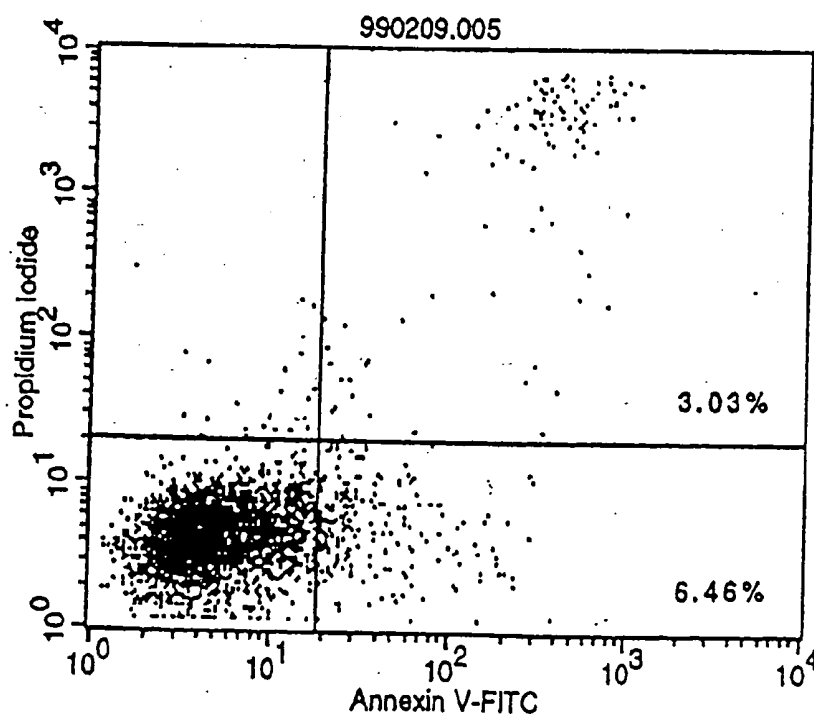


Fig. 16

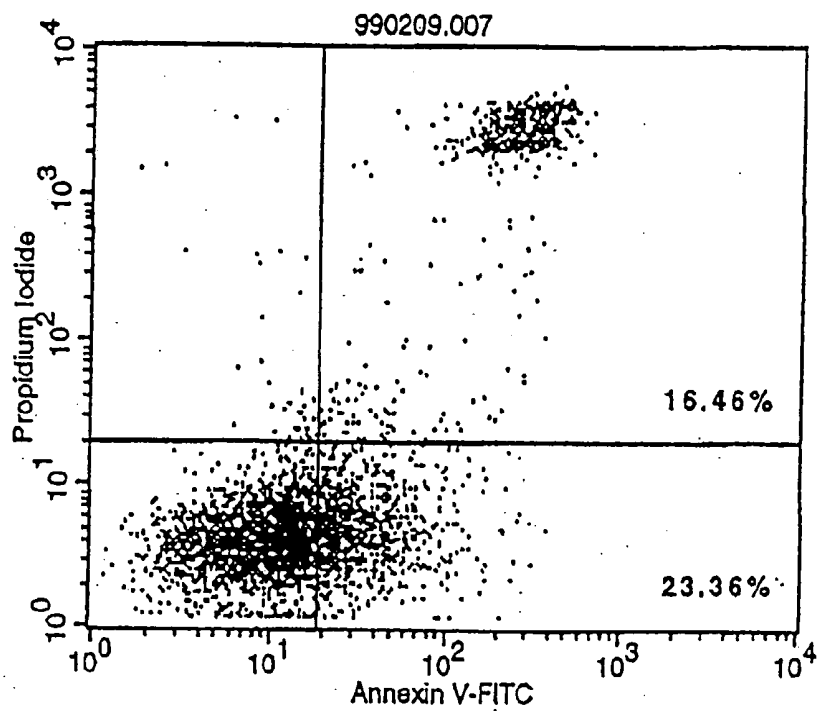


Fig. 17

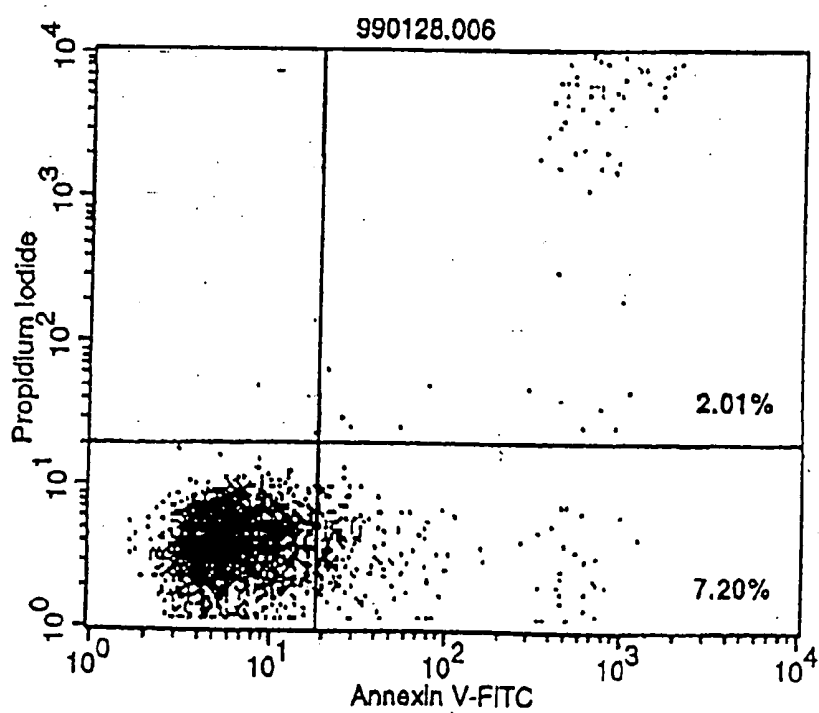


Fig. 18

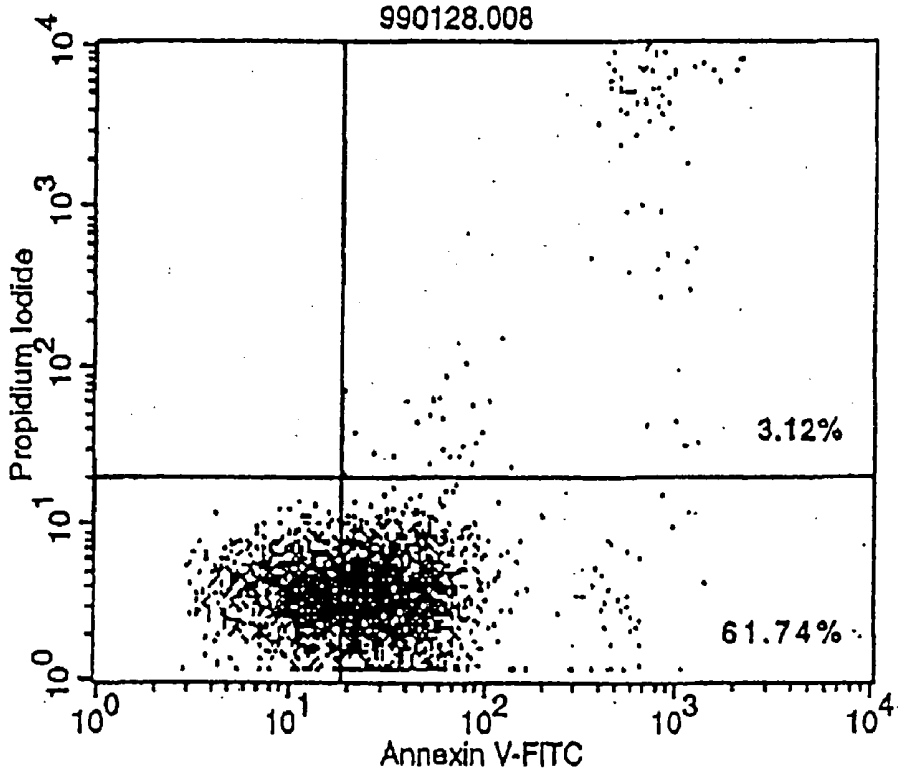


Fig. 19

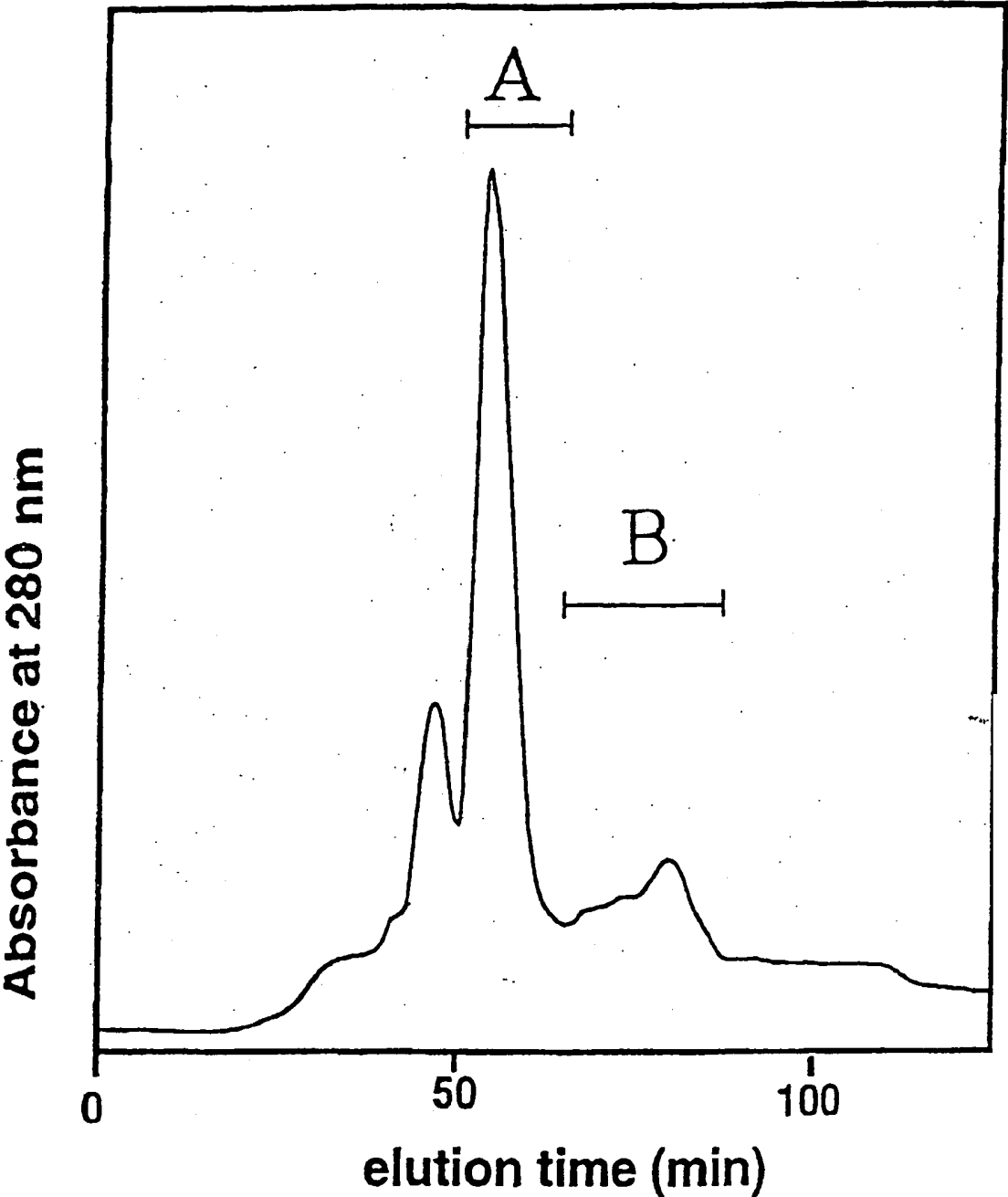


Fig. 20

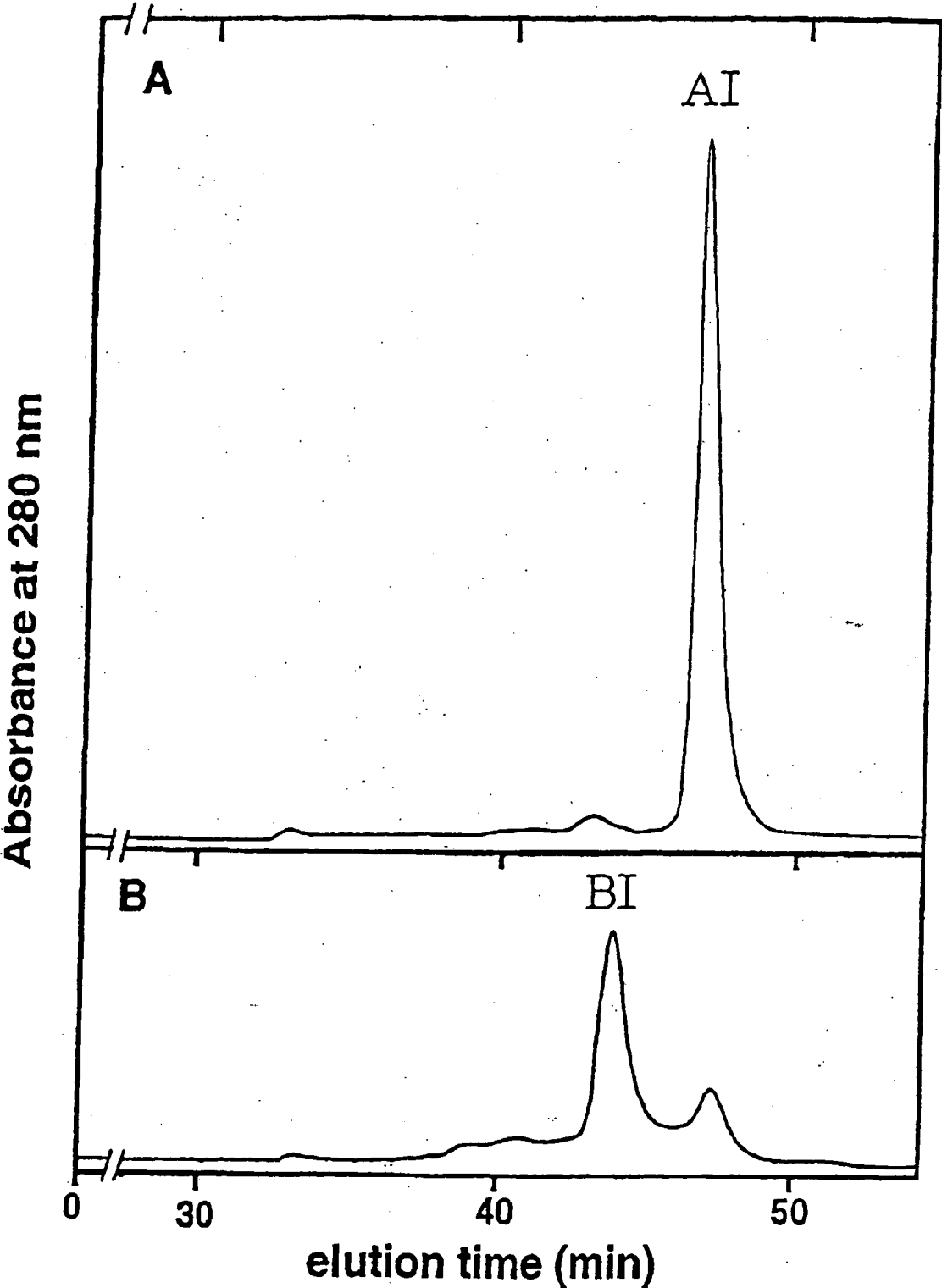


Fig. 21

SDS-PAGE analysis of MABL2-scFv

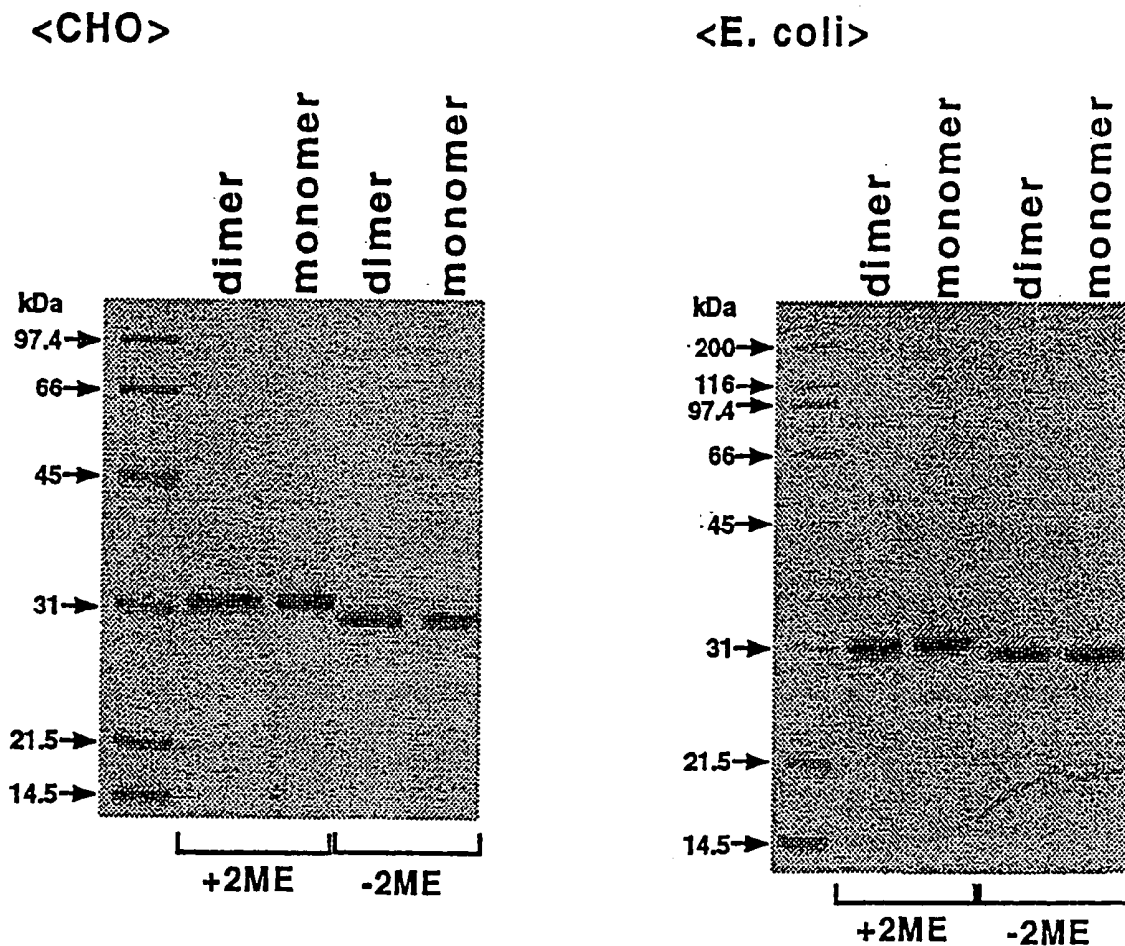


Fig. 22

TSK gel G3000SW
20 mM Acetate buffer, 0.15 M NaCl, pH 6.0

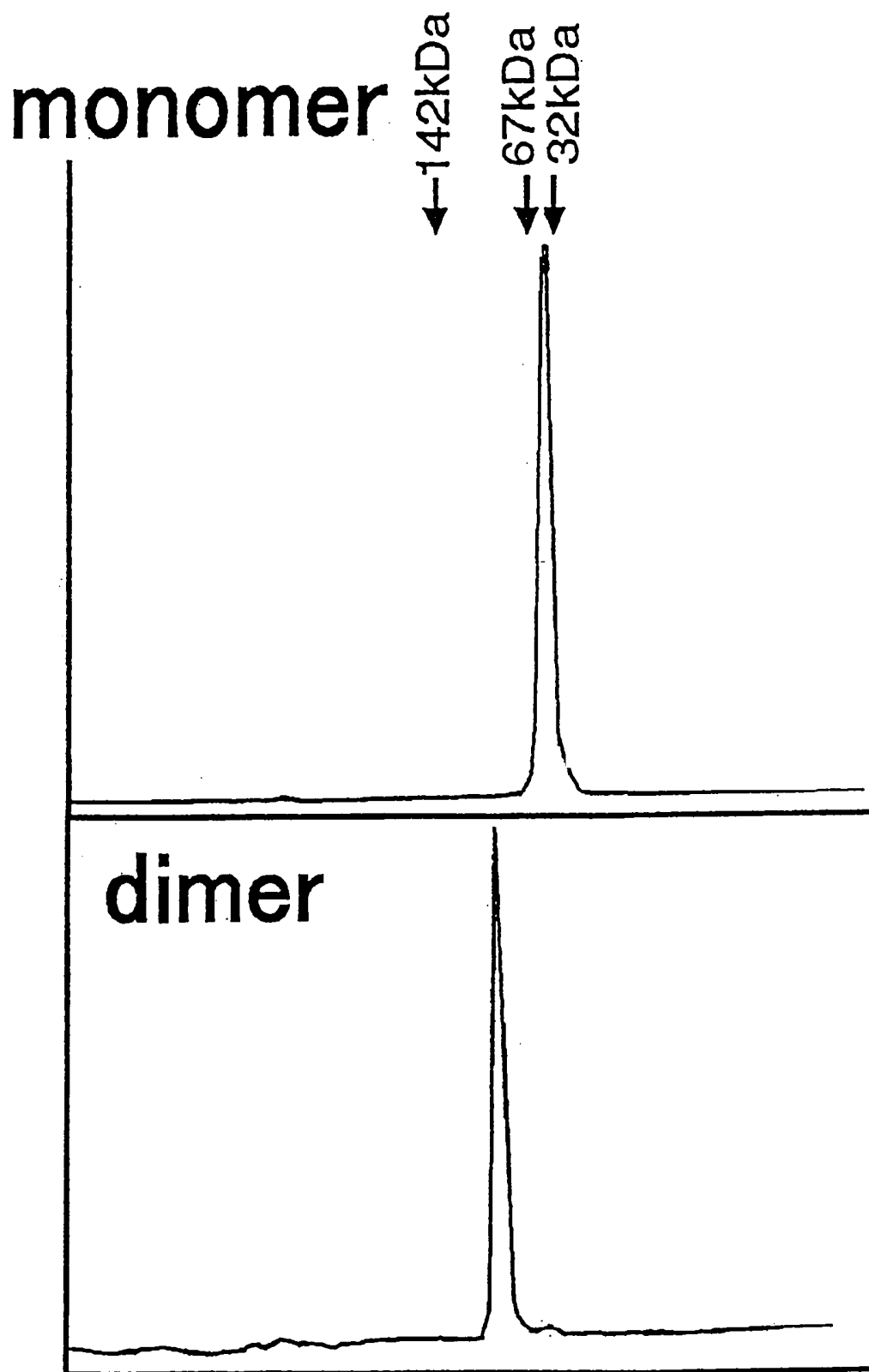


Fig. 23

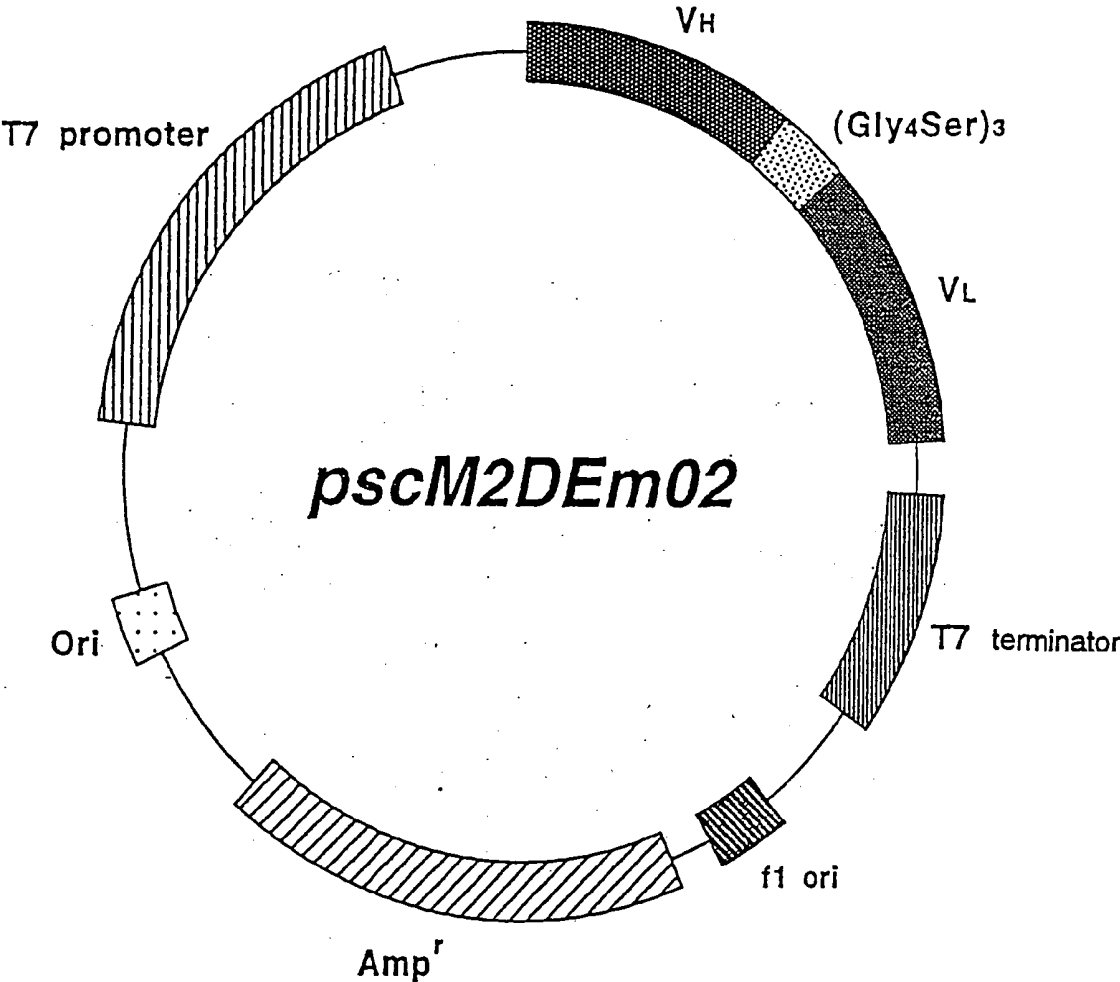


Fig. 24

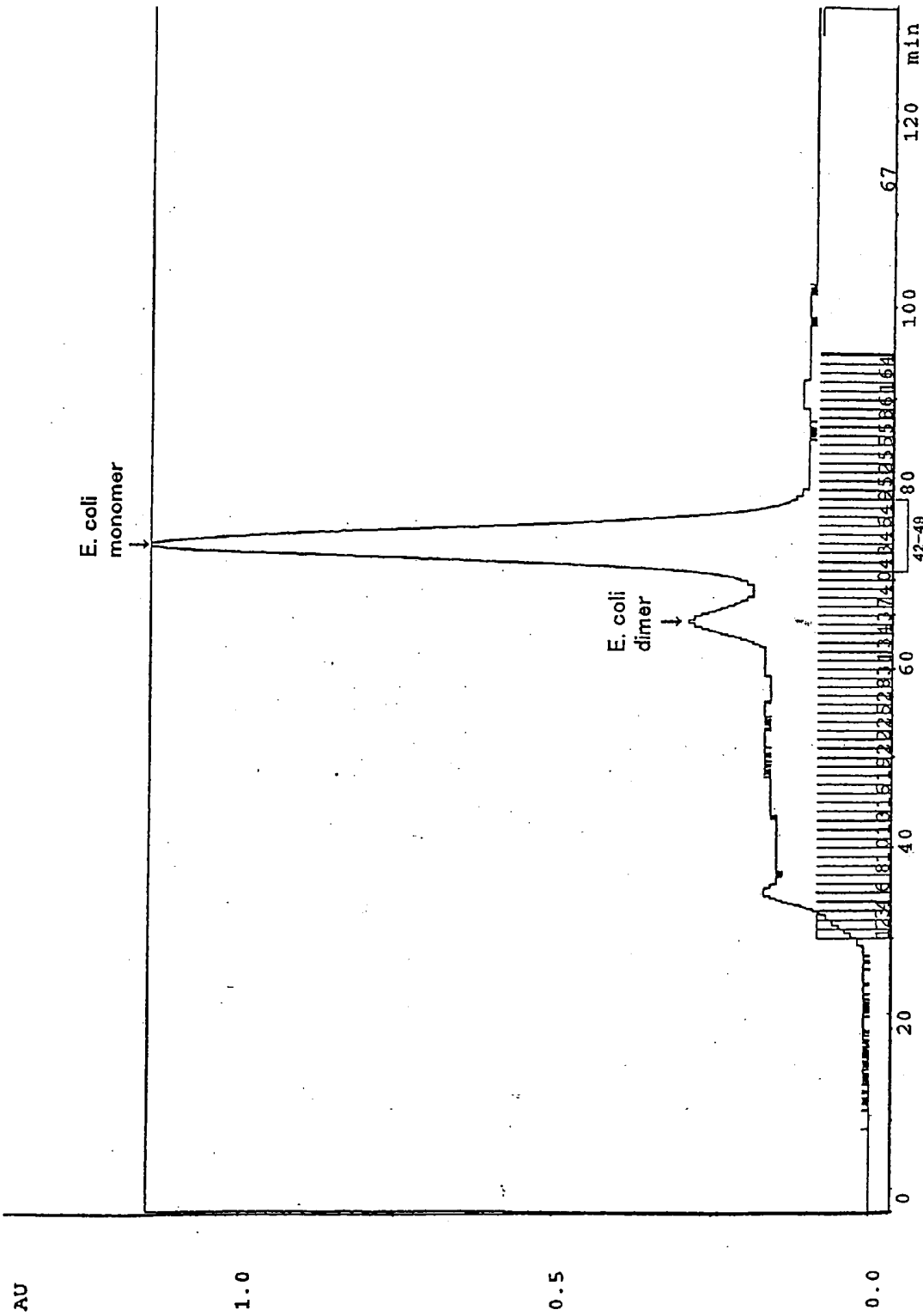


Fig. 25

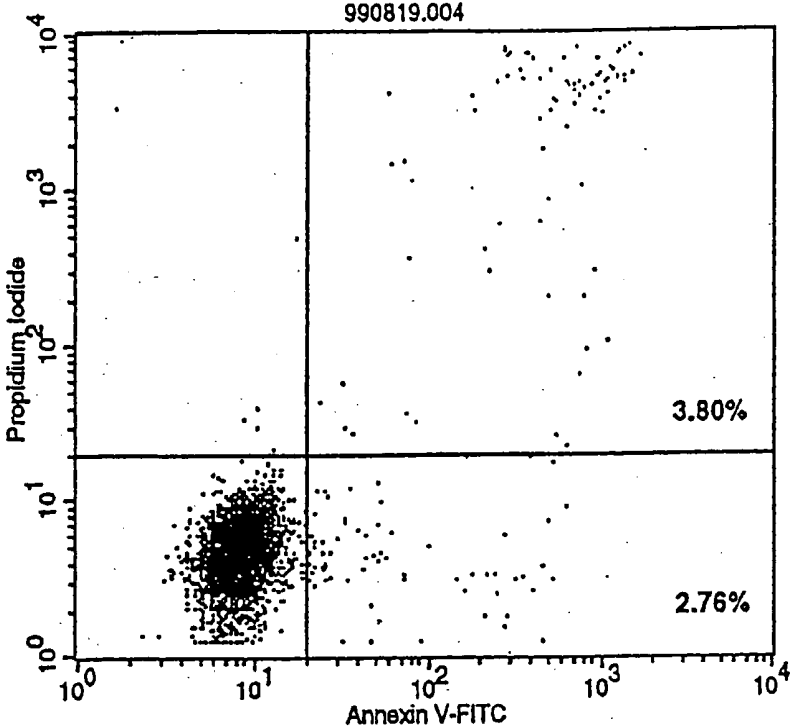


Fig. 26

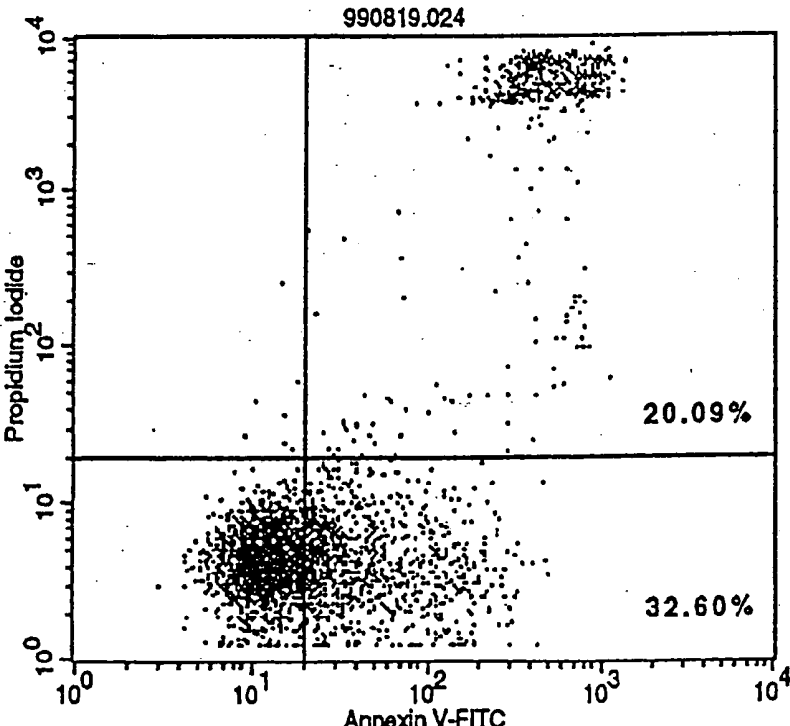


Fig. 27

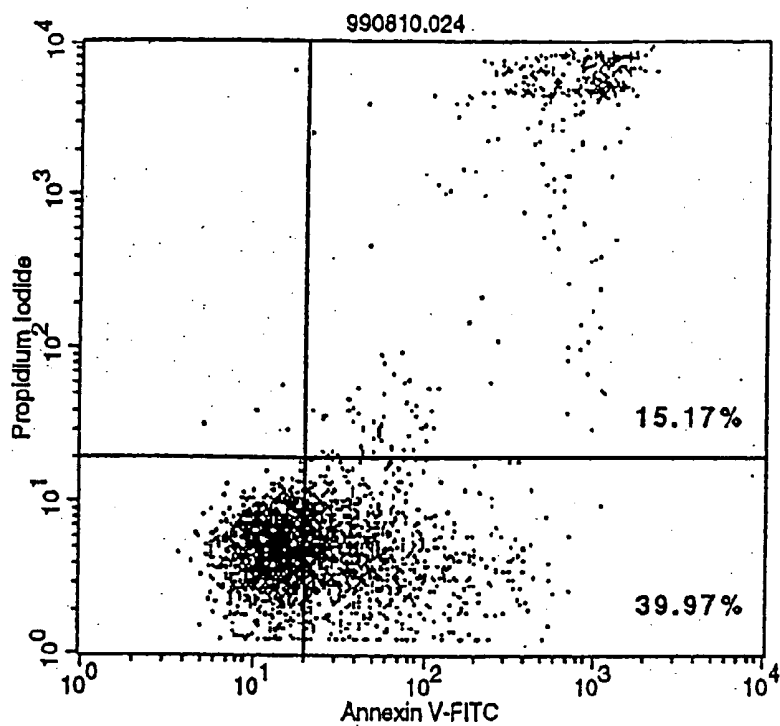


Fig. 28

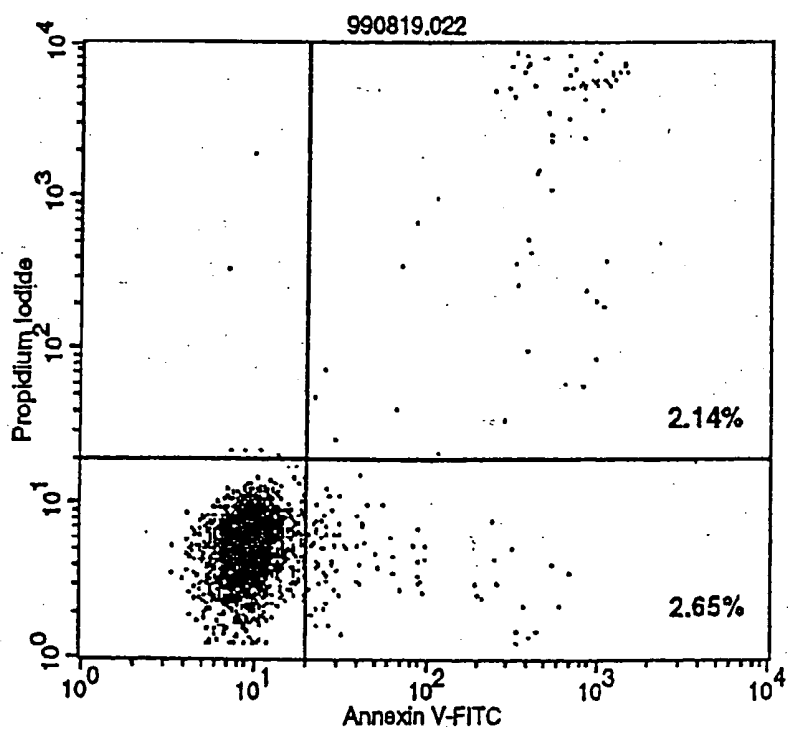


Fig. 29

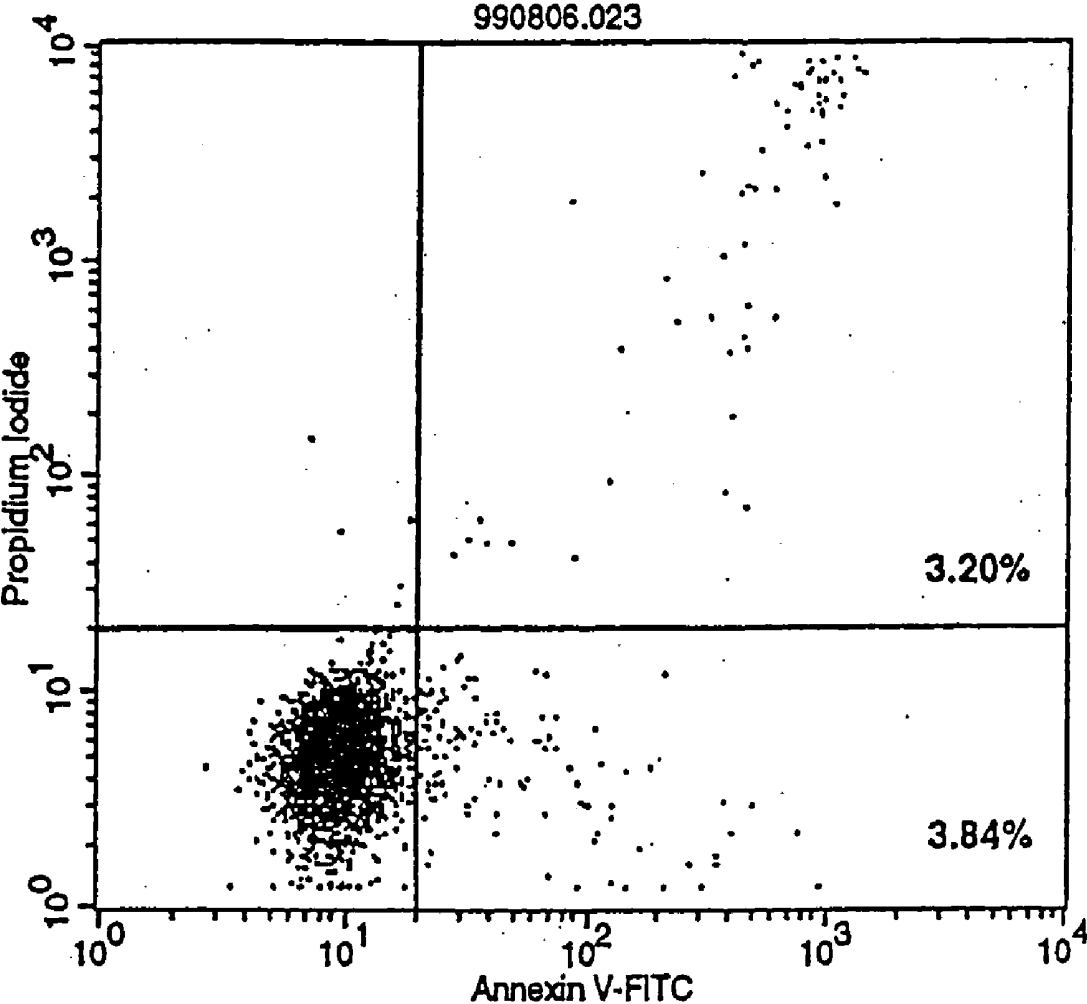


Fig. 30

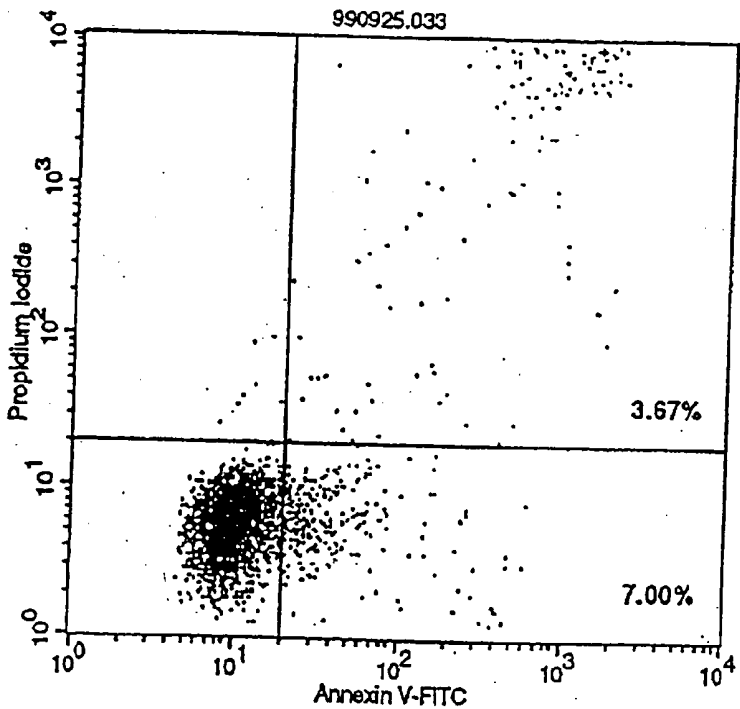


Fig. 31

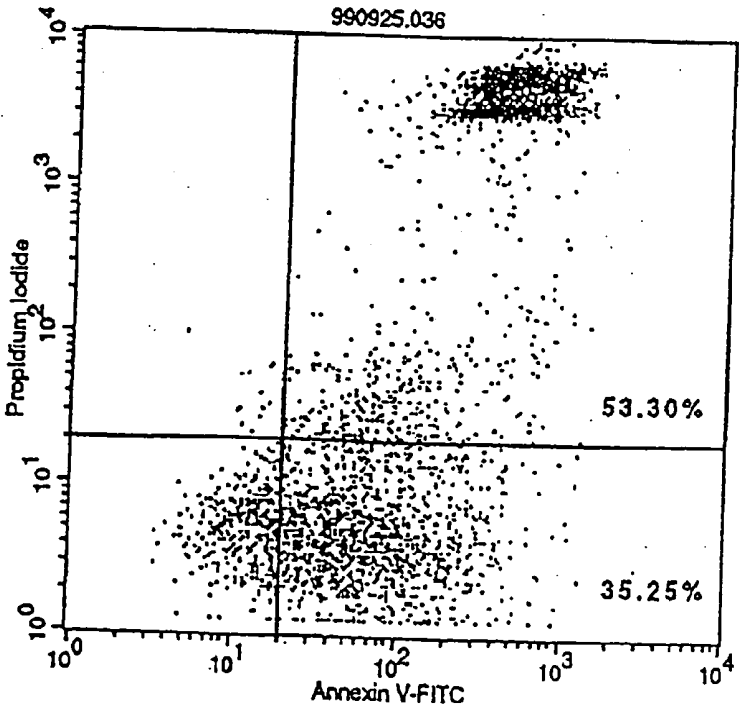
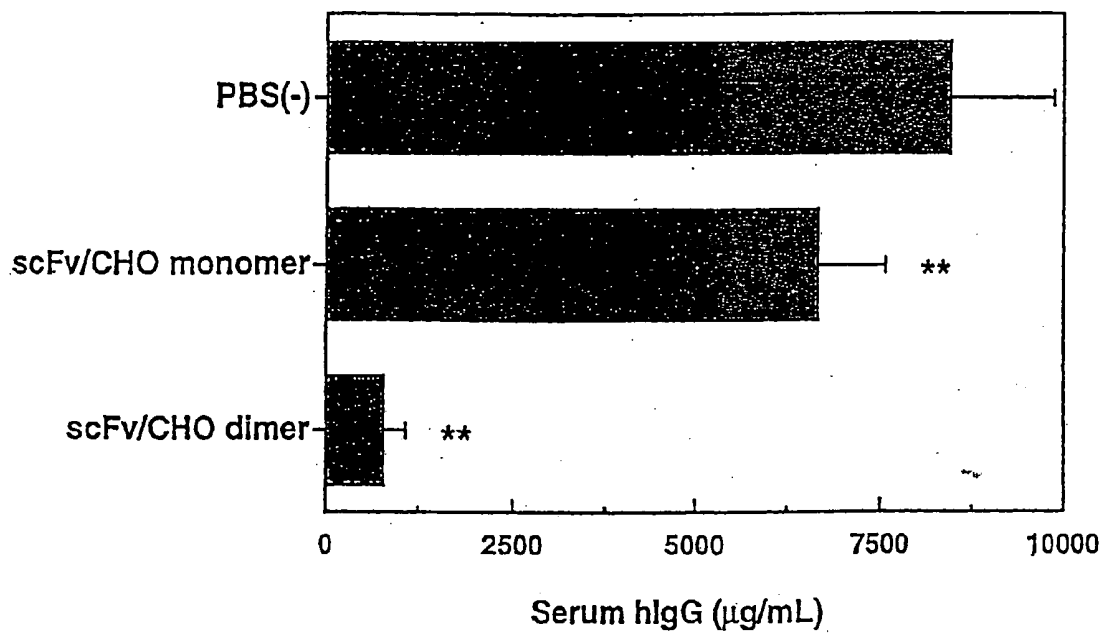


Fig. 32

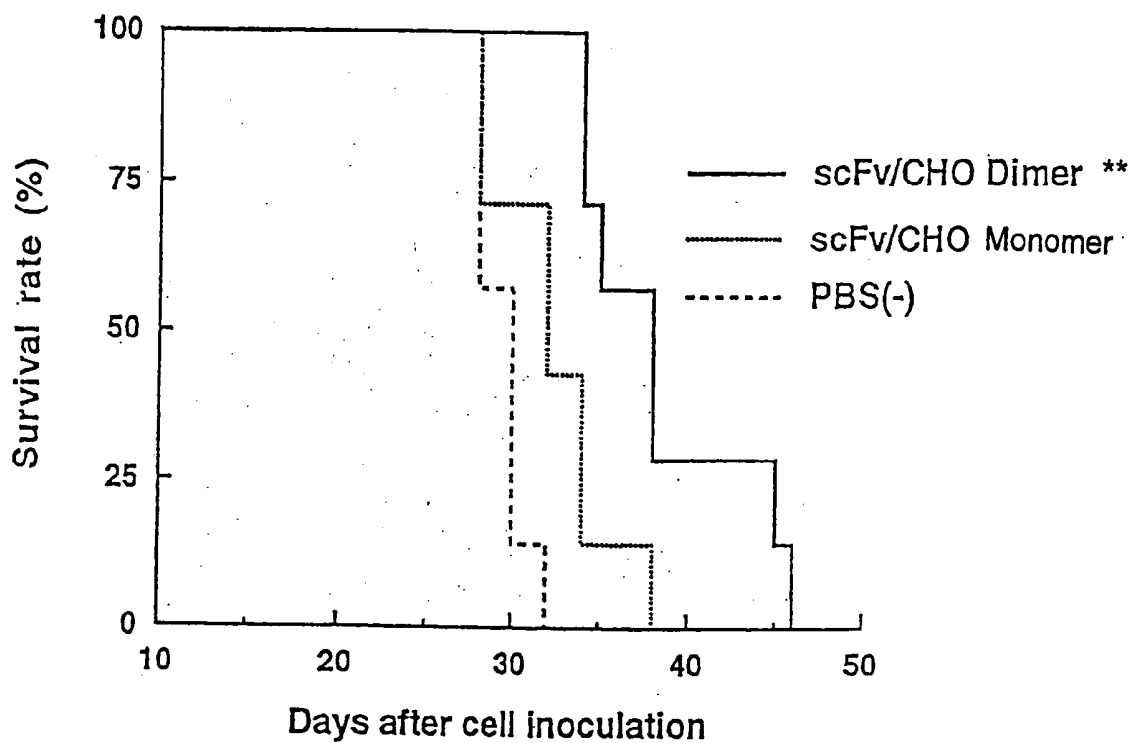
*Effect of MABL-2 (scFv) on serum hlgG
in KPMM2 i.v. SCID mice*



** : p<0.01

Fig. 33

Effect of MABL-2 (scFv) on survival of KPMM2 i.v. SCID mice



** ; P<0.01 by t-test

Fig. 34

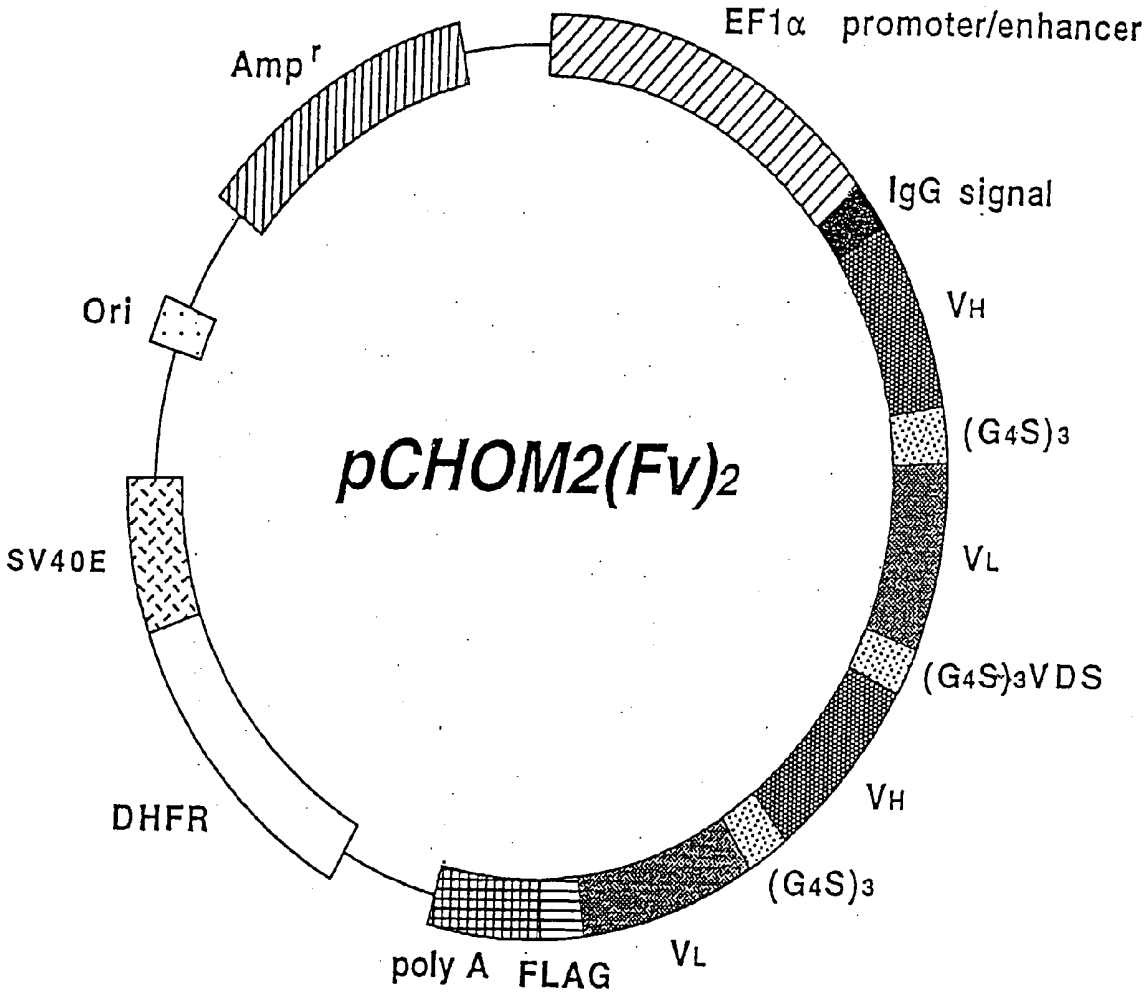


Fig. 35

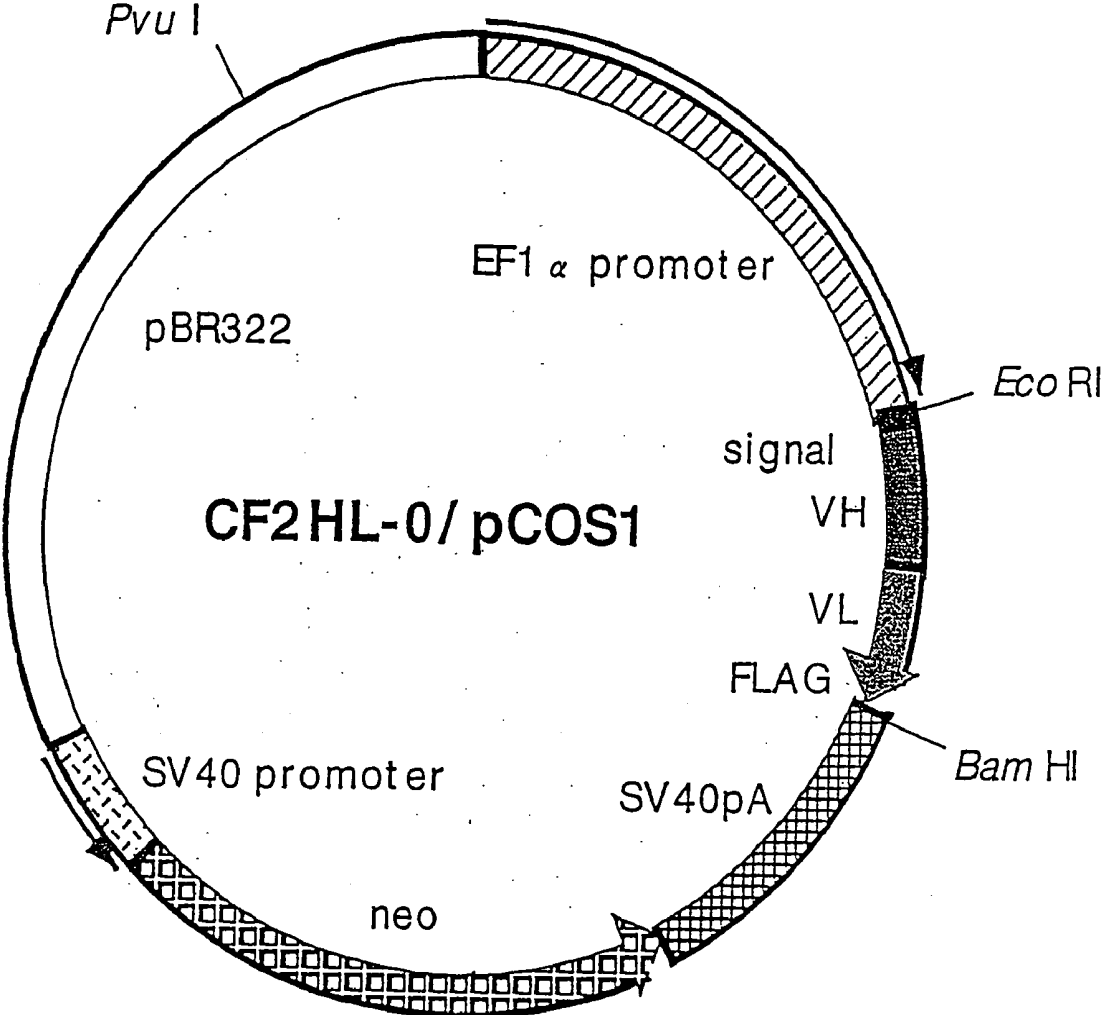
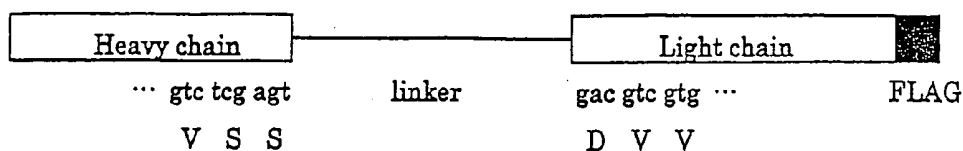


Fig. 36

Base Sequence and Amino Acid Sequence of Linker for HL Type



Plasmid	Number of	
	linker amino acid	linker
CF2HL-0/pCOS1	0	gtc tcg agt V S S
CF2HL-3/pCOS1	3	gtc tcg agt ggt ggt tcc V S S G G S
CF2HL-4/pCOS1	4	gtc tcg agt ggt ggt ggt tcc V S S G G G S
CF2HL-5/pCOS1	5	gtc tcg agt ggt ggt ggt ggt tcc V S S G G G G S
CF2HL-6/pCOS1	6	gtc tcg agt gt ggt ggt ggt ggt tcc V S S G G G G G S
CF2HL-7/pCOS1	7	gtc tcg agt ggt ggt ggt ggt ggt ggt tcc V S S G G G G G G S

Fig. 37

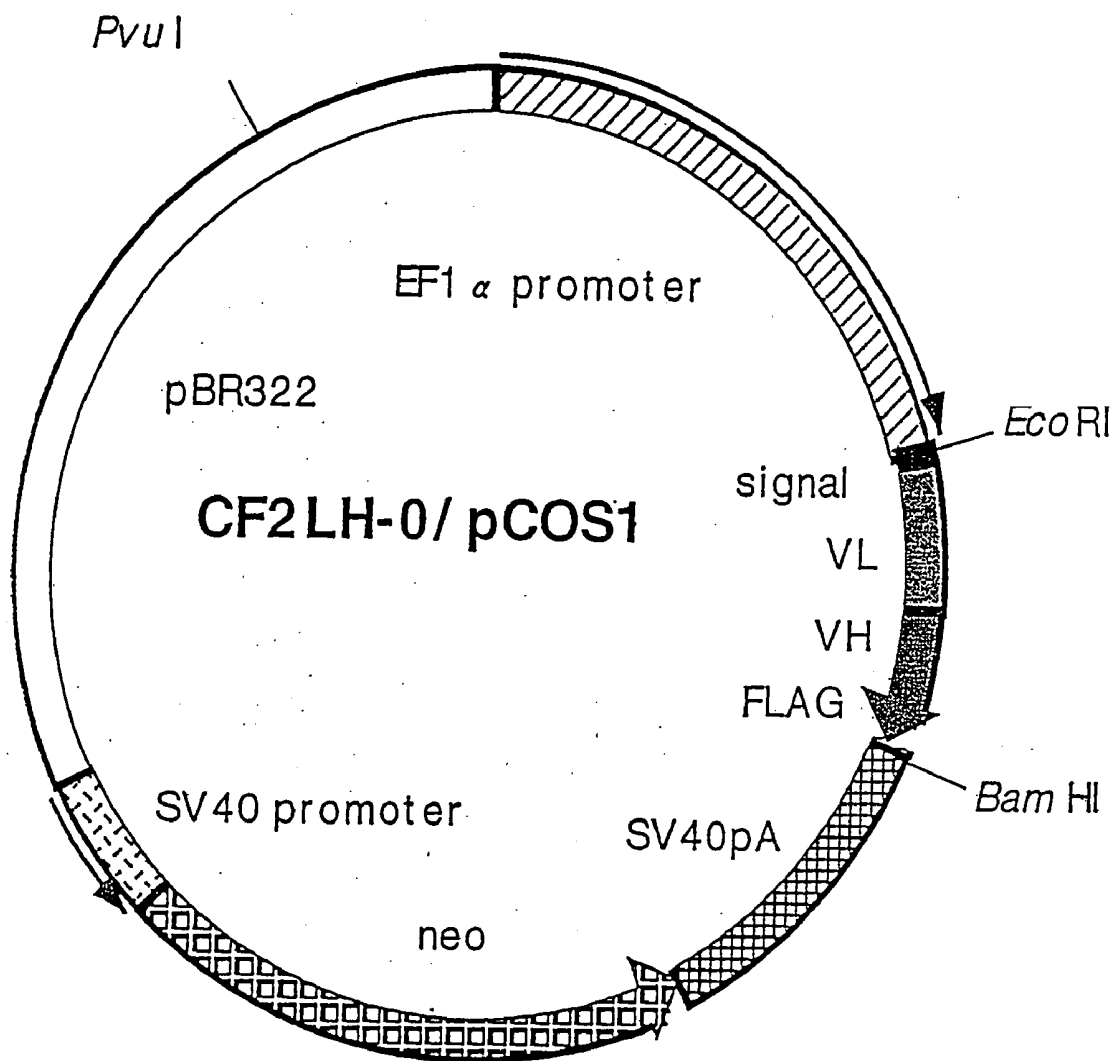
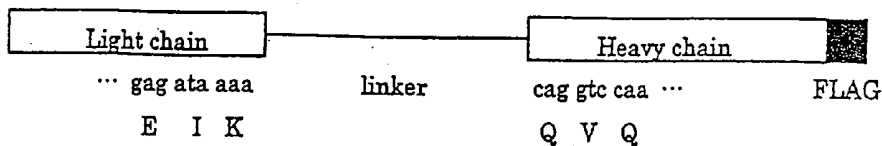


Fig. 38

Base Sequence and Amino Acid Sequence of Linker for LH Type



Plasmid	Number of	
	linker amino acid	linker
CF2LH-0/pCOS1	0	gag ata aaa E I K
CF2LH-3/pCOS1	3	gag ata aaa tcc gga ggc E I K S G G
CF2LH-4/pCOS1	4	gag ata aaa tcc gga ggt ggc E I K S G G G
CF2LH-5/pCOS1	5	gag ata aaa tcc gga ggt ggt ggc E I K S G G G G
CF2LH-6/pCOS1	6	gag ata aaa tcc gga ggt ggt ggt ggc E I K S G G G G G
CF2LH-7/pCOS1	7	gag ata aaa tcc gga ggt ggt ggt ggt ggc E I K S G G G G G G

Fig. 39

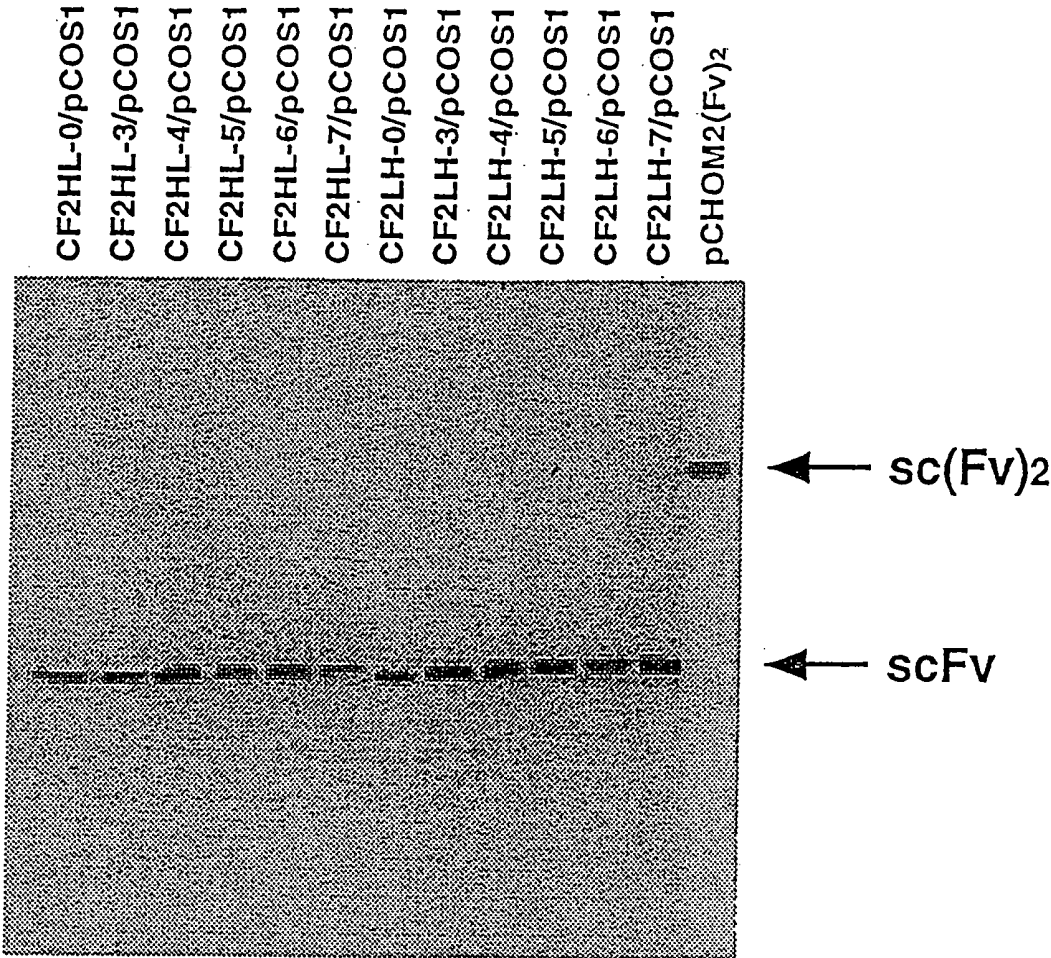


Fig. 40a

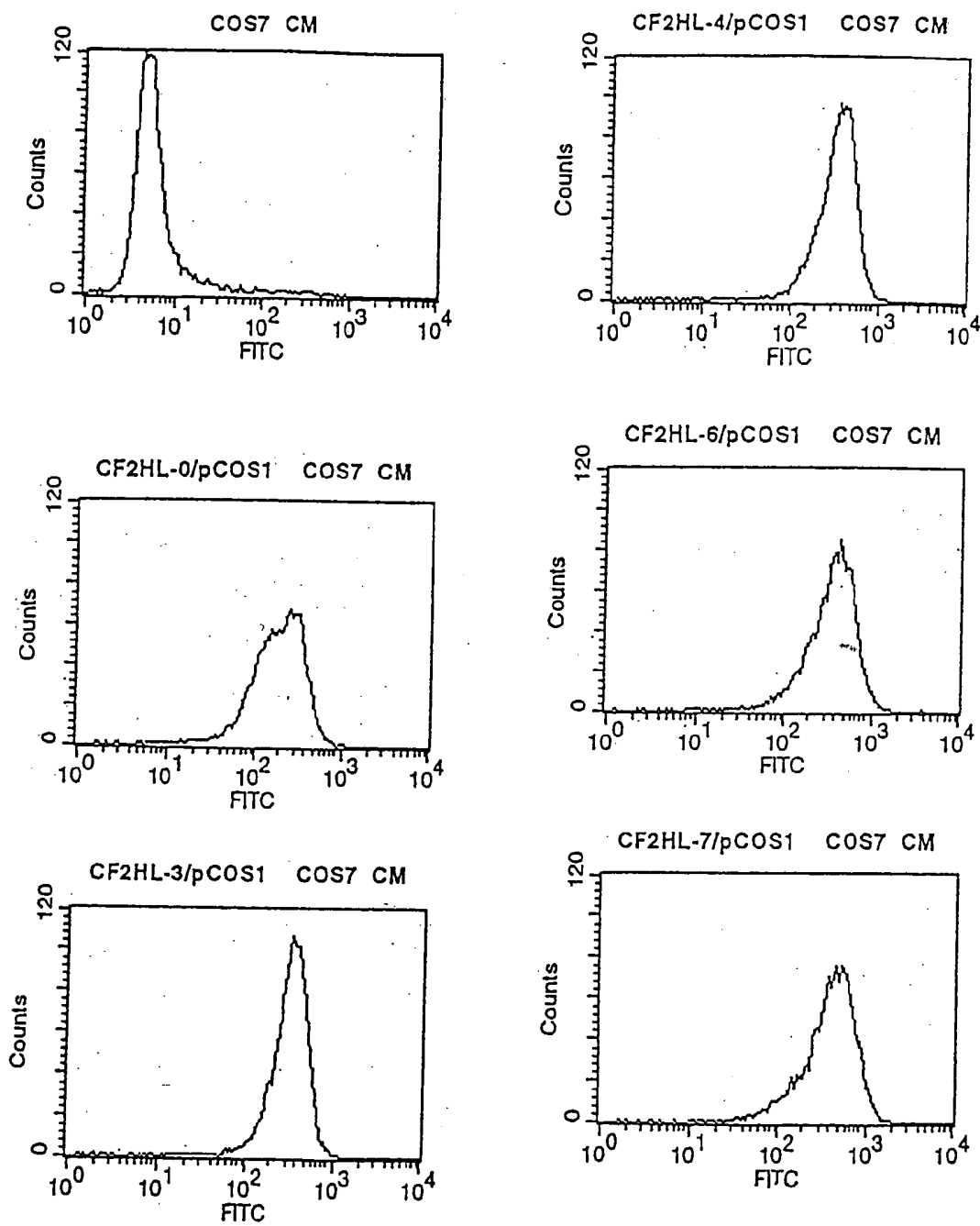


Fig. 40b

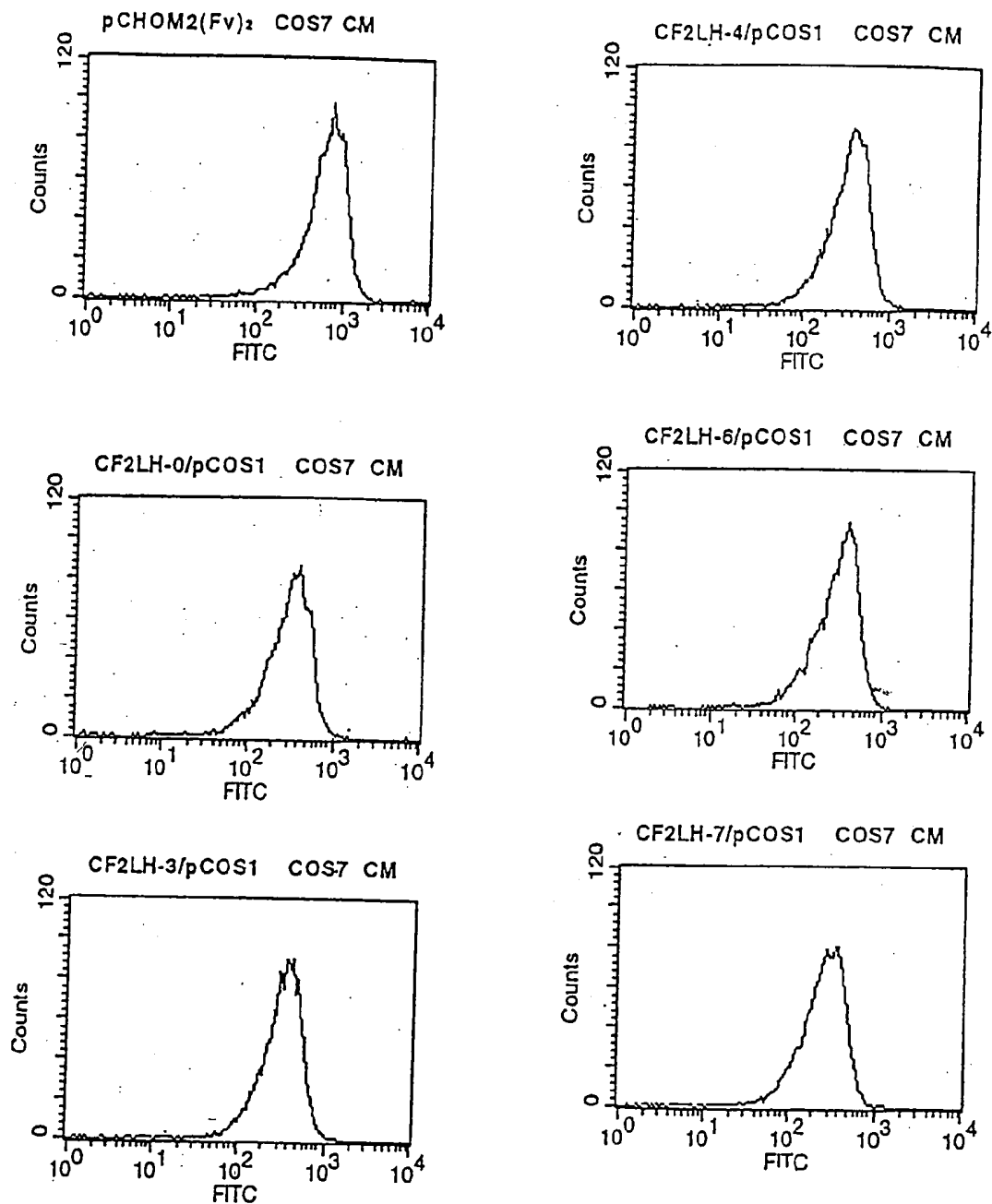


Fig. 41

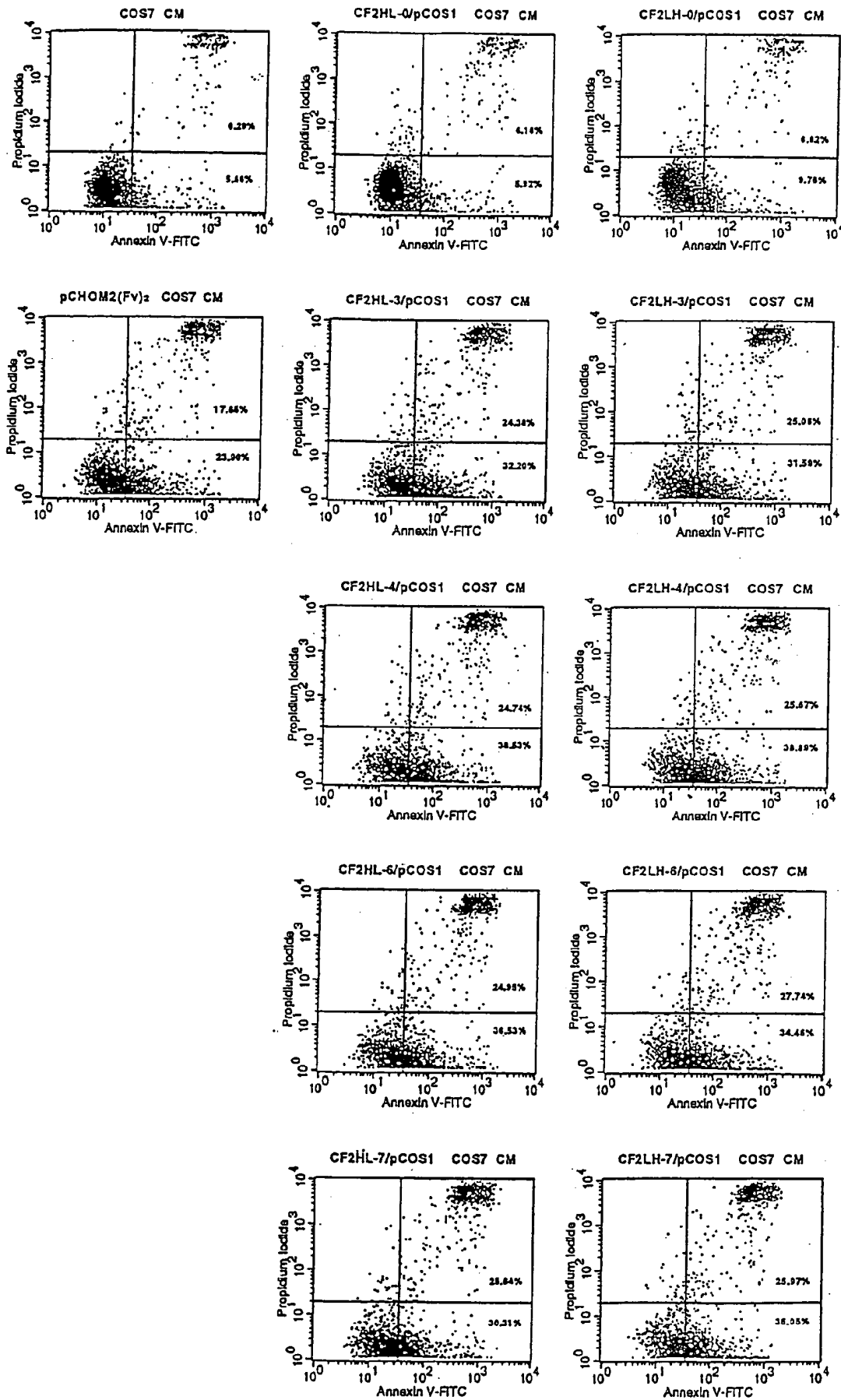


Fig. 42

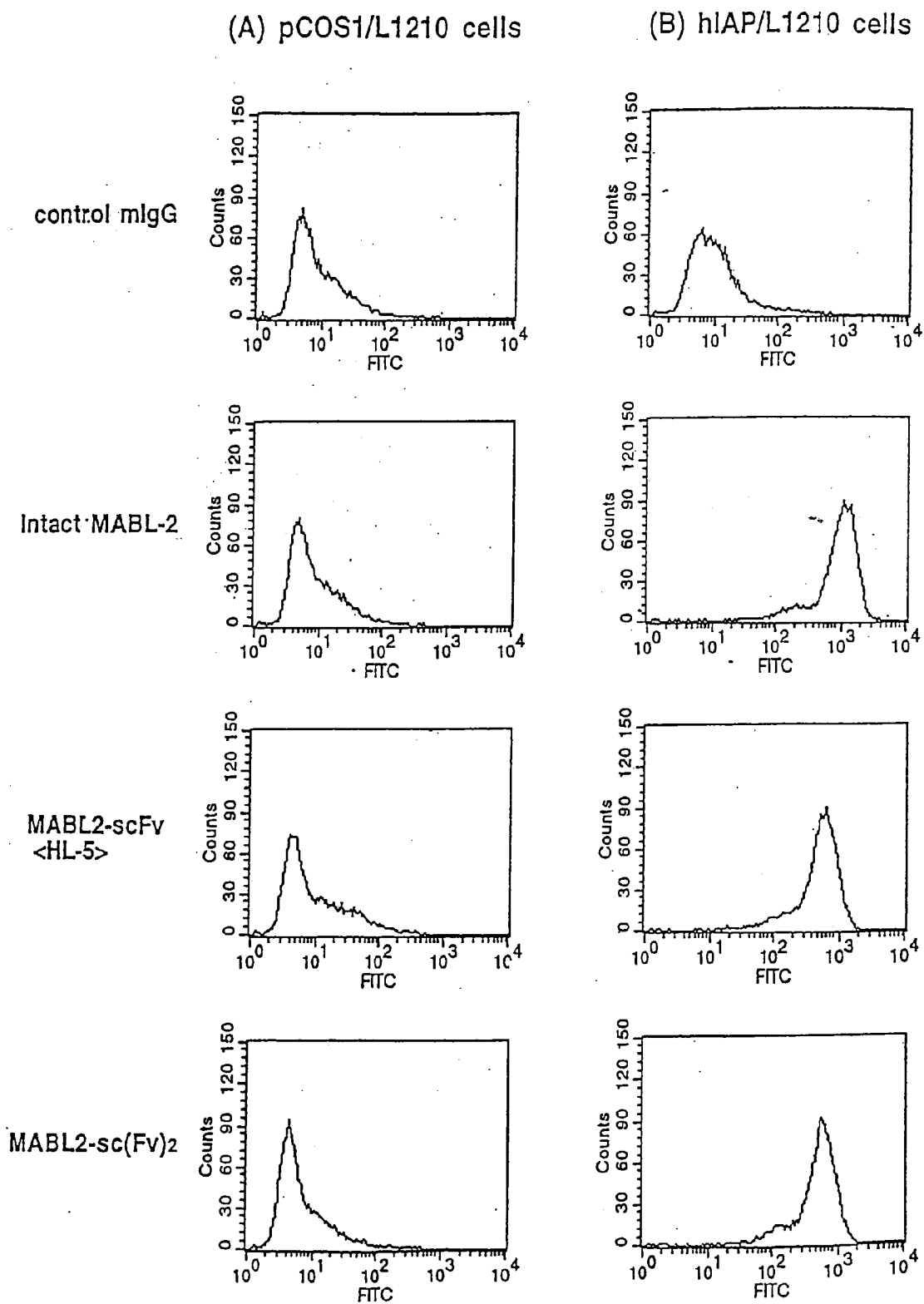


Fig. 43

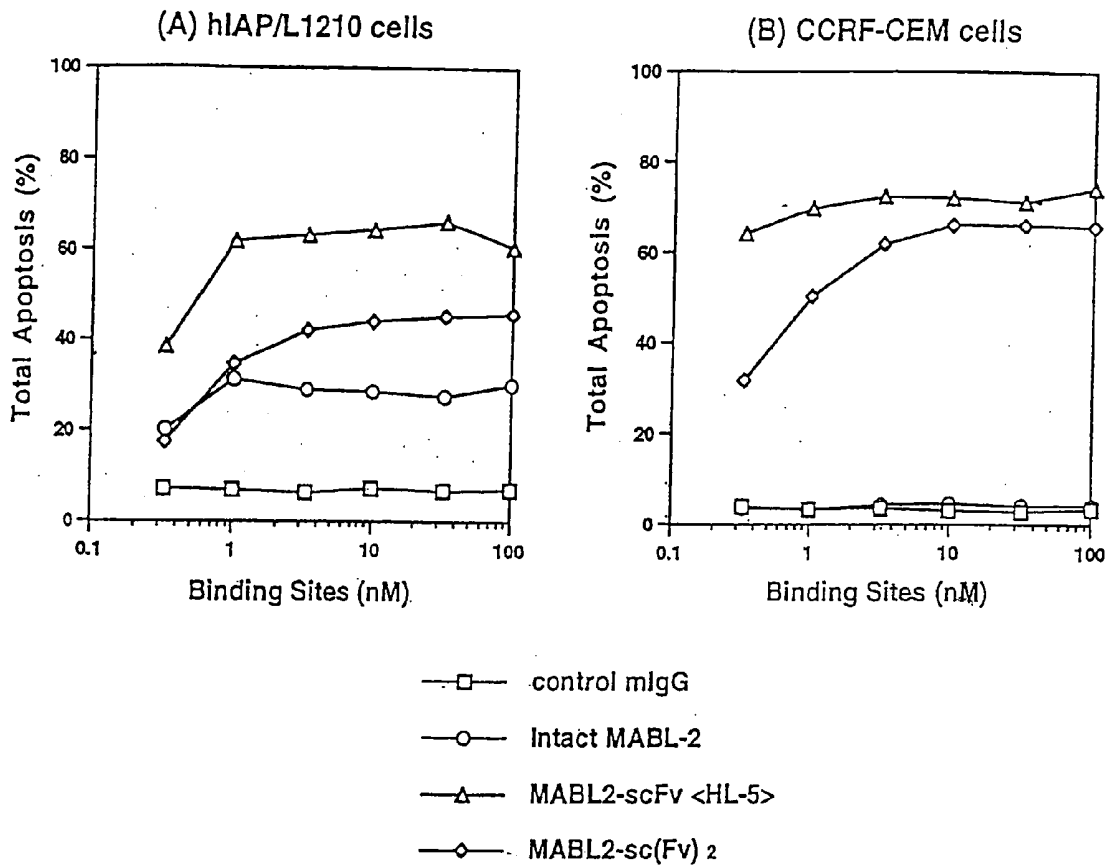


Fig. 44

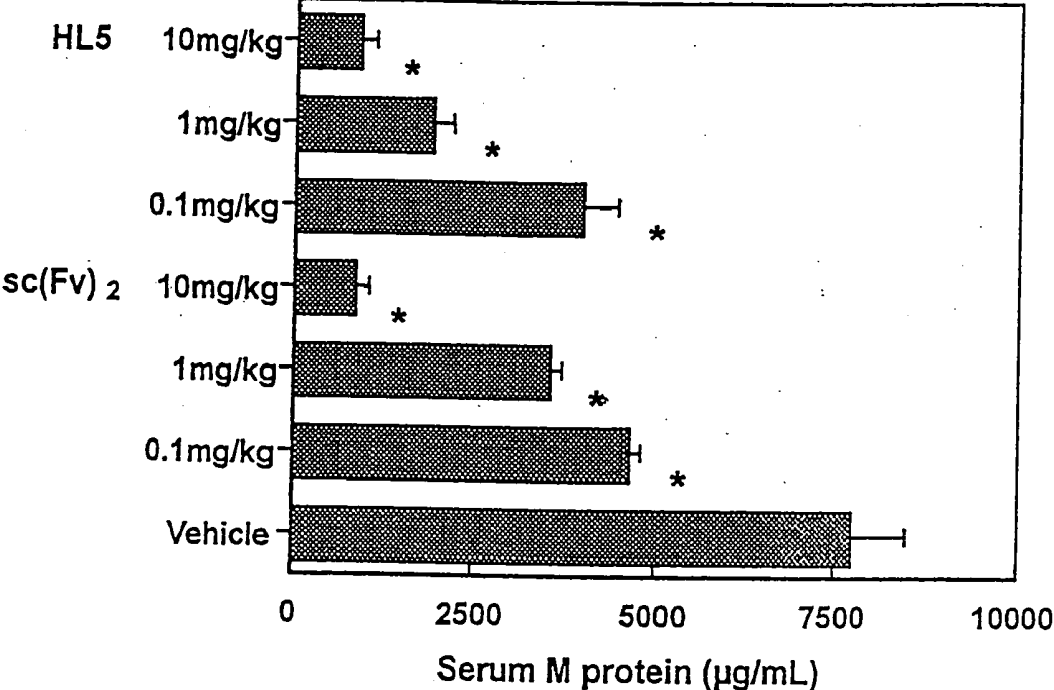


Fig. 45

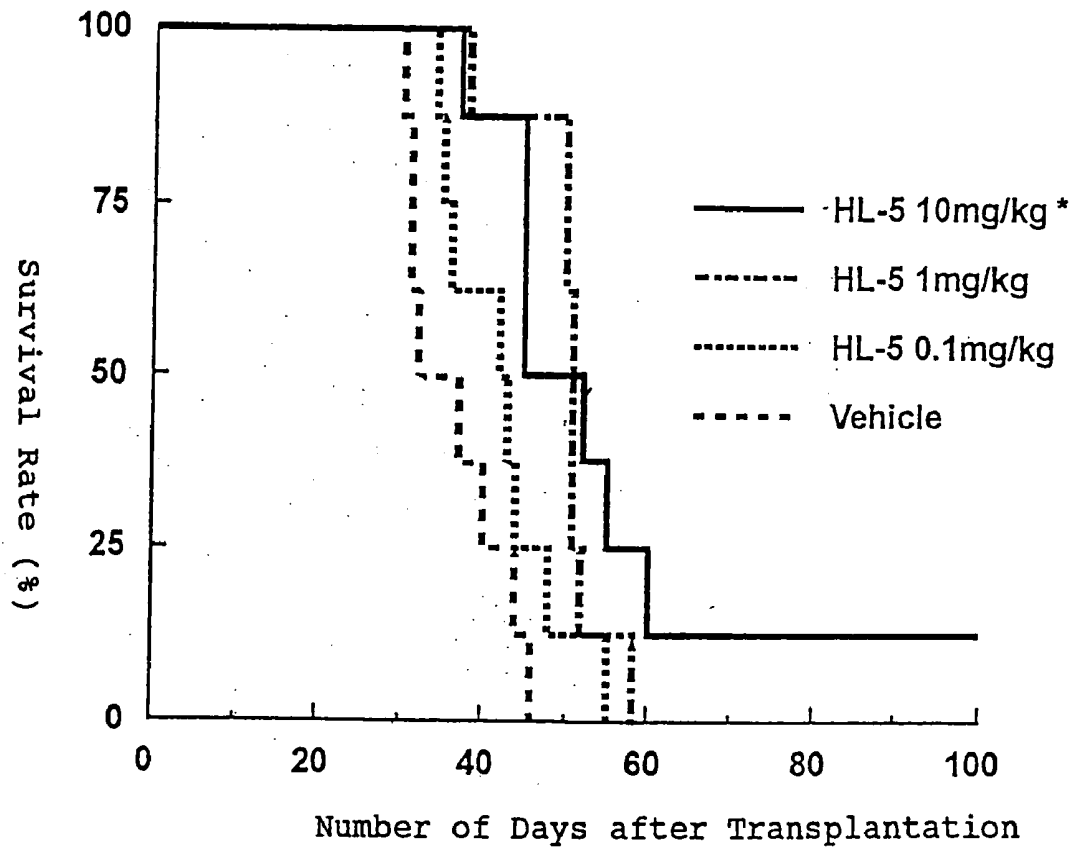


Fig. 46

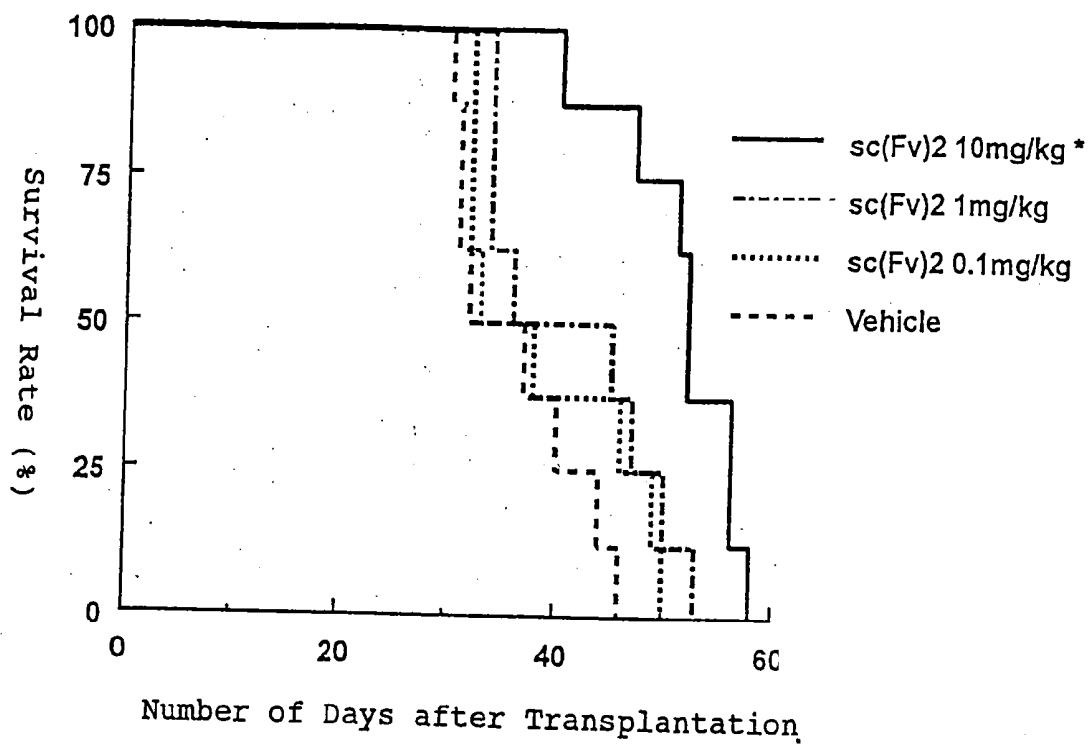


Fig. 47

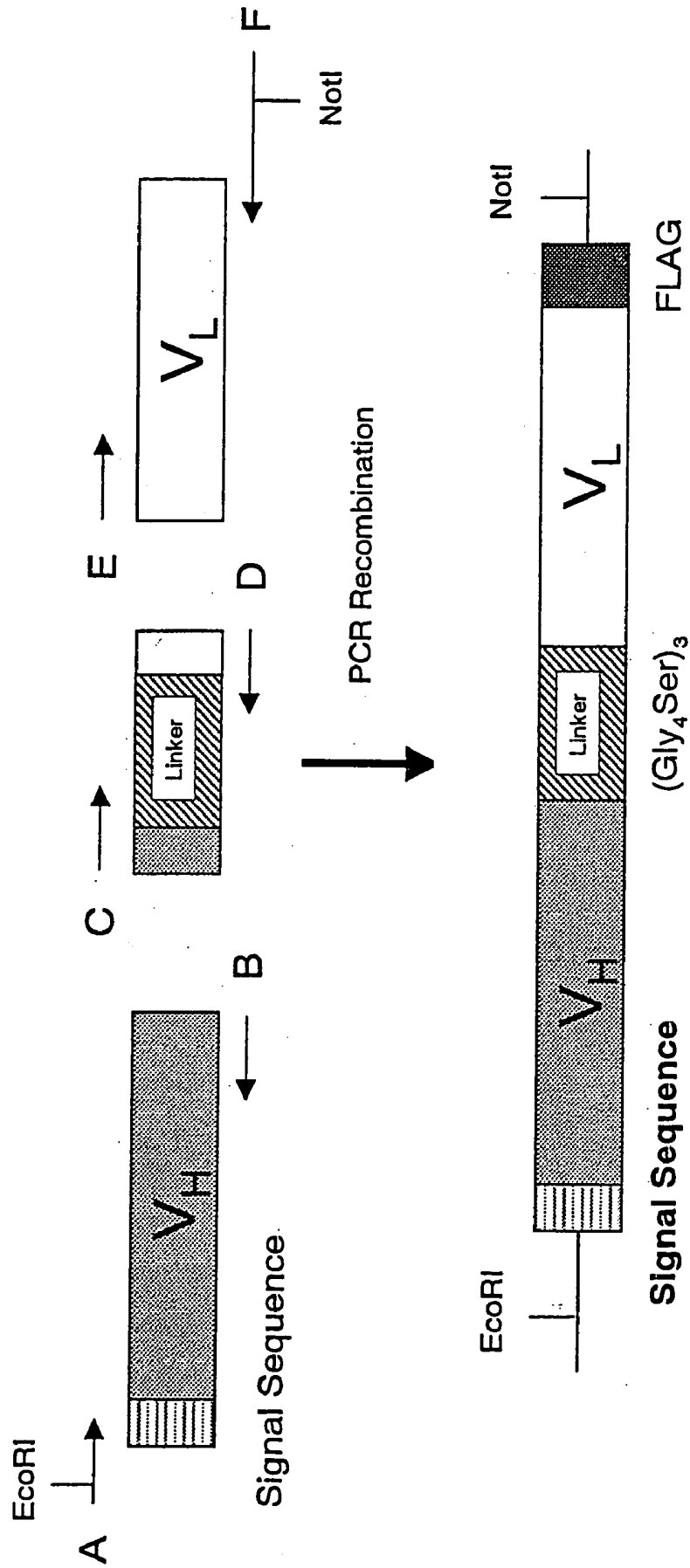


Fig. 48

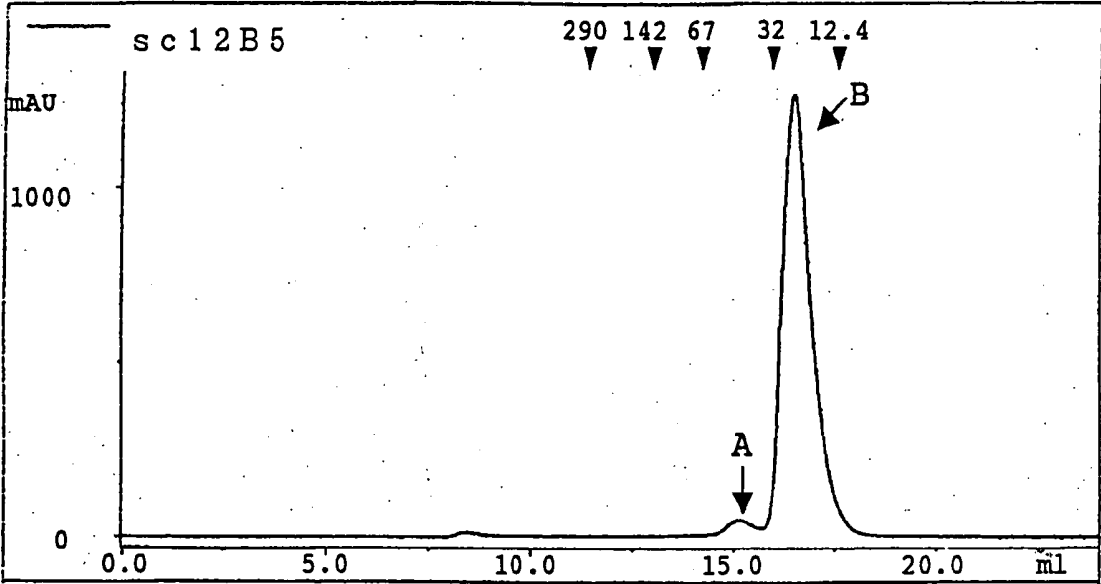
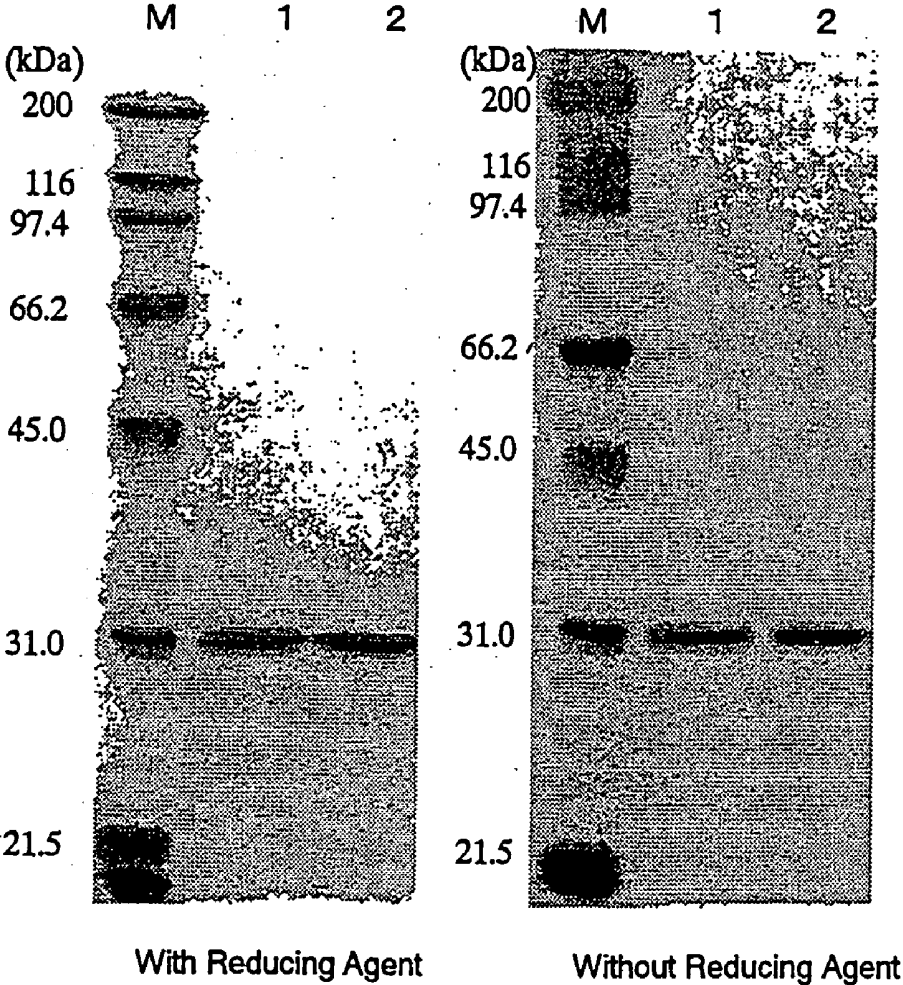


Fig. 49



M:MW marker
1:sc12B5 fractionA
2:sc12B5 fractionB

Fig. 50

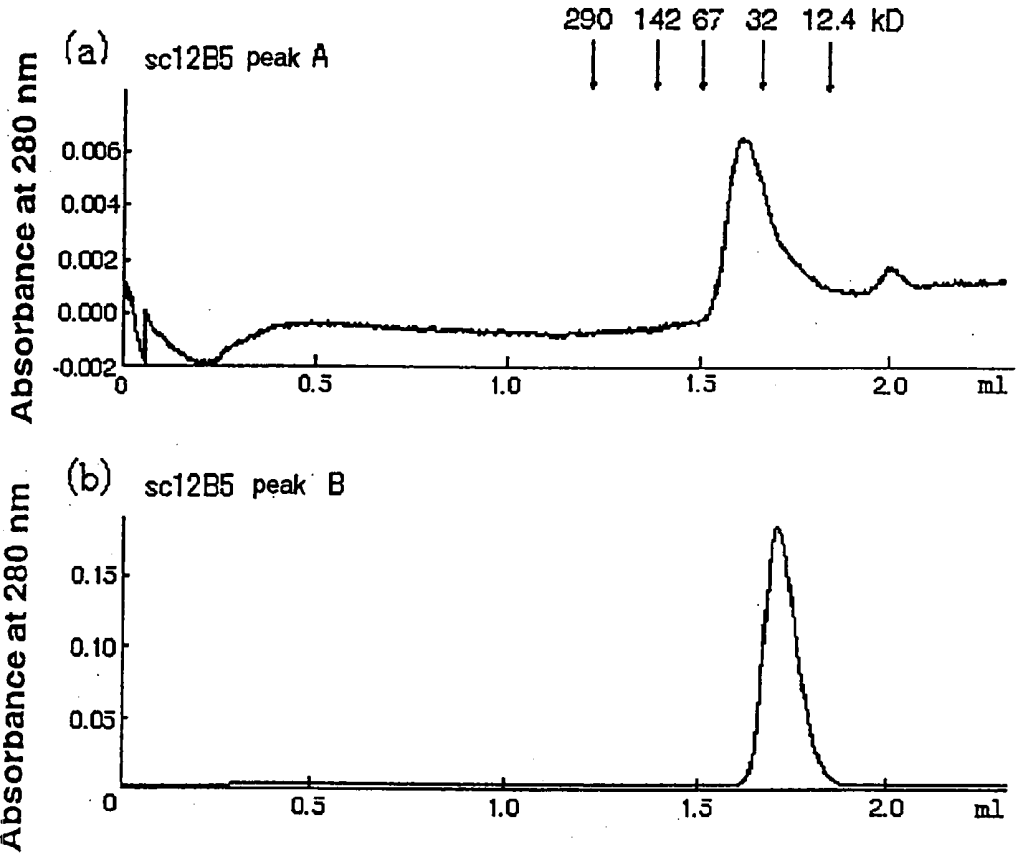


Fig. 51

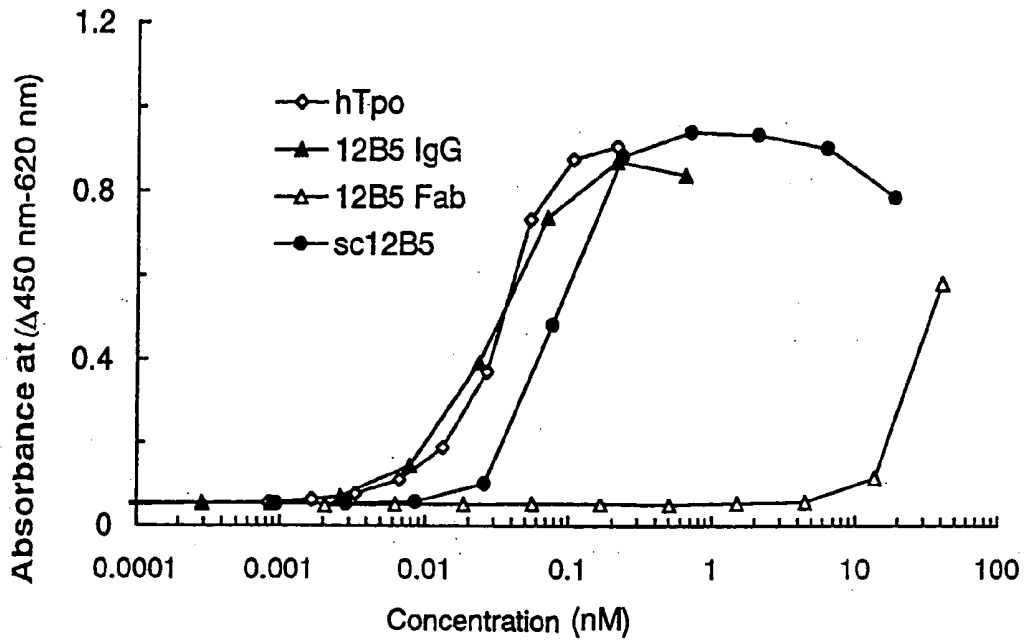
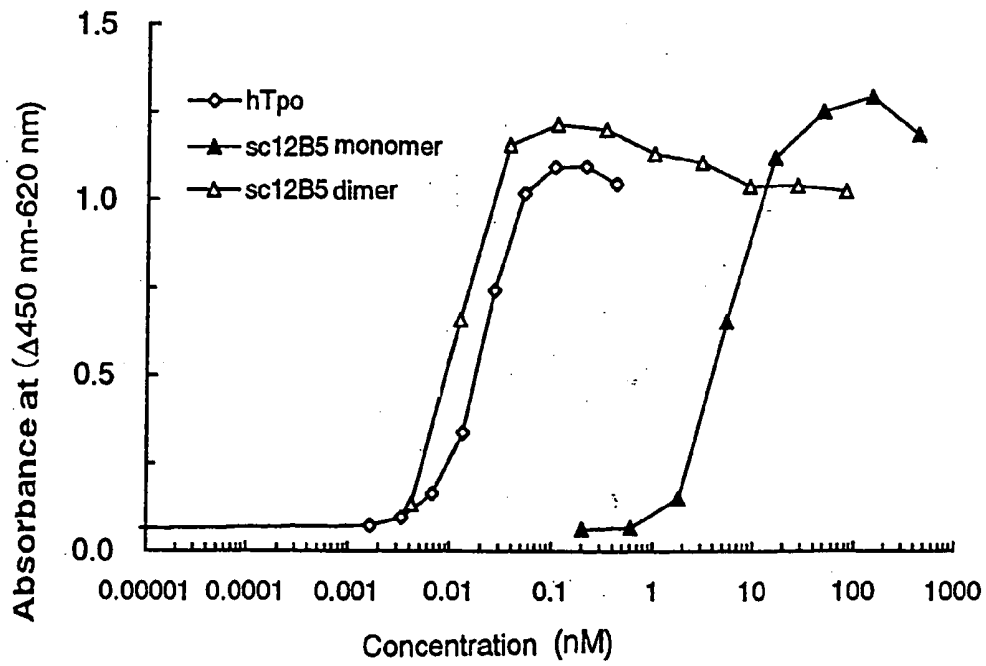


Fig. 52



AGONIST ANTIBODIES

TECHNICAL FIELD

[0001] This invention relates to modified antibodies containing two or more H chain V regions and two or more L chain V regions of a monoclonal antibody which show agonist activity by crosslinking a cell surface molecule(s). The modified antibodies have agonist activity of transducing a signal into cells by crosslinking a cell surface molecule(s) which can transduce a signal into cells and useful as a medicine for various purposes.

BACKGROUND ART

[0002] JP-A 9-295999 discloses the preparation of a specific monoclonal antibody using a splenic stromal cell line as a sensitizing antigen aiming at developing specific antibodies that can recognize the aforementioned splenic stromal cells and the preparation of novel monoclonal antibodies that recognize mouse Integrin Associated Protein (mouse IAP) as an antigen. JP-A. 9-295999 also discloses that the monoclonal antibodies are capable of inducing apoptosis of myeloid cells.

[0003] WO99/12973 discloses monoclonal antibodies whose antigen is human Integrin Associated Protein (hereinafter referred to as human IAP; amino acid sequence and nucleotide sequence thereof are described in *J. Cell Biol.*, 123, 485-496, 1993; see also *Journal of Cell Science*, 108, 3419-3425, 1995) and which are capable of inducing apoptosis of human nucleated blood cells (myeloid cell and lymphocyte) having said human IAP. These monoclonal antibodies are referred to antibody MABL-1 and antibody MABL-2, and hybridomas producing these antibodies are also referred to MABL-1 (FERM BP-6100) and MABL-2 (FERM BP-6101), respectively.

[0004] Japanese Patent Application 11-63557 describes the preparation of single chain Fvs having single chain Fv regions from the monoclonal antibodies whose antigen is human IAP. The single chain Fvs are capable of inducing apoptosis of nucleated blood cells having human IAP.

[0005] The monoclonal antibody recognizing IAP as an antigen induces apoptosis of nucleated blood cells having human IAP, but it also causes hemagglutination in vitro. It indicates that the administration of a large amount of the monoclonal antibody recognizing IAP as an antigen may result in a side effect such as hemagglutination.

[0006] The inventors made intensive research for utilizing the monoclonal antibodies against human IAP as therapeutic agent of blood diseases and obtained single chain Fvs having the single chain Fv region capable of inducing apoptosis of nucleated blood cells having human IAP.

[0007] On the other hand modified antibodies, especially antibodies with lowered molecular size, for example, single chain Fvs were developed to improve permeability into tissues and tumors by lowering molecular size and to produce by a recombinant method. Recently the dimers of single chain Fvs, especially hetero-dimers are used for crosslinking cells. They are bispecific modified antibodies, whose typical example is hetero-dimers of single chain Fvs recognizing antigens of cancer cells and antigens of host cells like NK cells and neutrophils (Kipriyanov et al., *Int. J. Cancer*, 77, 9763-9772, 1998). They were produced by

construction technique of single chain Fv as modified antibodies, which are more effective in treating cancers by inducing intercellular crosslinking. It has been thought that the intercellular crosslinking is induced by antibodies and their fragments (e.g. Fab fragment), bispecific modified antibodies and even dimers of single chain Fvs, which are monospecific.

[0008] As antibodies capable of transducing a signal by crosslinking a cell surface molecule(s), there are known an antibody against EPO receptor involved in cell differentiation and proliferation (JP-A 2000-95800), an antibody against MuSK receptor (Xie et al., *Nature Biotech.* 15, 768-771, 1997) and others. However there have been no reports on modified antibodies with lowered molecular size.

[0009] Noticing that antibody MABL-1, antibody MABL-2 and dimers derived from them induced apoptosis of cells having IAP, the inventors discovered that they crosslink (dimerize) IAP receptor on cell surface, thereby a signal is transduced into the cells and, as a result, apoptosis is induced. This suggests that monospecific single chain Fv dimers crosslink a cell surface molecule(s) (e.g. receptor) and transduce a signal like a ligand, thereby serving as an agonist. Focusing on the intercellular crosslinking, it was discovered that the above-mentioned single chain Fv dimers do not cause hemagglutination while the above-mentioned monoclonal antibodies do. The same result was also observed with single chain bivalent antibodies (single chain polypeptides containing two H chain V regions and two L chain V regions). This suggests that monoclonal antibodies may form intercellular crosslinking while modified antibodies like single chain Fv dimers and single chain bivalent antibodies crosslink a cell surface molecule(s) but do not form intercellular crosslinking.

[0010] Discovering that an antibody molecule (whole IgG) can be modified into single chain Fv dimers, single chain bivalent antibodies and the like which crosslink a cell surface molecule(s), thereby reducing side effects caused by intercellular crosslinking and providing new medicines inducing only desired effect on the cell, the inventors completed the invention. The modified antibodies have remarkably high activity compared with original monoclonal antibodies and improved permeability into tissues due to the characteristics of having lower molecular size compared with the original antibodies and of having no constant regions.

DISCLOSURE OF INVENTION

[0011] An object of this invention is to provide low molecular-size agonist modified antibodies which contain two or more H chain V regions and two or more L chain V regions of a monoclonal antibody and which combine with a cell surface molecule(s) and transduce a signal into cells, thereby can serve as an agonist.

[0012] Therefore, this invention relates the modified antibodies which include two or more H chain V regions and two or more L chain V regions, preferably 2 to 4 each, especially preferably two each, and show an agonist activity by crosslinking a cell surface molecule(s).

[0013] Preferable examples of the modified antibodies of the invention are dimers of the single chain Fv which contains one H chain V region and one L chain V region, or

a single chain polypeptide containing two H chain V regions and two L chain V regions. The H chain V region and L chain V region are preferably connected through a linker in the modified antibodies.

[0014] The above-mentioned single chain Fv dimer includes a dimer by non-covalent bond, a dimer by a covalent bond through a crosslinking radical and a dimer through a crosslinking reagent (an antibody, an antibody fragment, or bivalent modified antibody). Conventional crosslinking radicals used for crosslinking peptides can be used as the crosslinking radicals to form the dimers. Examples are disulfide crosslinking by cysteine residue, other crosslinking radicals such as C₄-C₁₀ alkylene (e.g. tetramethylene, pentamethylene, hexamethylene, heptamethylene and octamethylene, etc.) or C₄-C₁₀ alkenylene (cis/trans-3-butenylene, cis/trans-2-pentenylene, cis/trans-3-pentenylene, cis/trans-3-hexenylene, etc.).

[0015] Moreover, the crosslinking reagent which can combine with a single chain Fv is, for example, an amino acid sequence which can optionally be introduced into Fv, for example, an antibody against FLAG sequence and the like or a fragment thereof, or a modified antibody originated from the antibody, for example, single chain Fv.

[0016] The invention also relates to a method of inducing an agonist action to cells by administering the first ligand and the second ligand which combine with a cell surface molecule(s), and administering a substance which combine with the first and the second ligands and crosslink the first and second ligands. The first ligand and the second ligand can be any things which can induce an agonist action by being crosslinked. Preferable examples are monovalent modified antibodies, such as the same or different single chain Fv monomer, a fragment of antibody etc. The substance to crosslink the above-mentioned ligand can be any things that induce an agonist action to the cells by crosslinking the first ligand and the second ligand. Preferable examples are antibodies, fragments of antibodies, (Fab)₂ or bivalent modified antibodies. Examples of bivalent antibodies are (Fab)₂, dimers of single chain Fv containing one H chain V region and one L chain V region and single chain polypeptides containing two H chain V regions and two L chain V regions. The method is effective for exploring receptors that transduce a signal into cells by crosslinking, is expected to be employed for DDS to deliver a medicine to target cells and is also useful as a drug administration system which suppresses side effect and allows a medicine to become effective at desired time and for desired period.

[0017] The modified antibodies of this invention can be any things which contain L chain V region and H chain V region of monoclonal antibody (e.g. antibody MABL-1, antibody MABL-2) and which specifically recognize the cell surface molecule(s), for example, a protein (a receptor or a protein involved in signal transduction), or a sugar chain of the above-mentioned protein or of a cell membrane protein and crosslink said cell surface molecule(s), thereby transduce a signal into cells. Modified antibodies in which a part of amino acid sequence of V region has been altered are included.

[0018] The present invention also relates to the humanization of the above-mentioned modified antibodies. The humanized modified antibodies comprise a humanized H chain V region and/or a humanized L chain V region.

Specifically, the humanized modified antibodies consist of the humanized L chain V region which comprises a framework region (FR) derived from an L chain V region of human monoclonal antibody and an CDR derived from an L chain V region of mouse monoclonal antibody and/or the humanized H chain V region which comprises an FR derived from an H chain V region of human monoclonal antibody and a CDR derived from an H chain V region of mouse monoclonal antibody. In this case, the amino acid sequence of FR or CDR may be partially altered, e.g. deleted, replaced or added.

[0019] Furthermore, the present invention relates to polypeptides which comprise an L chain C region of human antibody and an L chain V region of the mouse monoclonal antibody, and/or an H chain C region of human antibody and an H chain V region of the mouse monoclonal antibody.

[0020] The present invention also relates to modified antibodies transducing a signal into cells by combining with cell surface molecule, thereby serving as an agonist, which comprise a CDR derived from a monoclonal antibody of other mammals than mouse (such as human, rat, bovine, sheep, ape and the like), which is equivalent to said mouse CDR, or an H chain V region and an L chain V region containing the CDR. Such CDRs, H chain V regions and L chain V regions may include CDRs derived from a human monoclonal antibody prepared from, for example, a transgenic mouse or the like, and H chain V regions and L chain V regions derived from a human monoclonal antibody containing the CDR.

[0021] The invention also relates to DNAs encoding the various modified antibodies as mentioned above and genetic engineering techniques for the producing recombinant vectors comprising the DNAs.

[0022] The invention also relates to host cells transformed with the recombinant vectors. Examples of host cells are animal cells such as human cells, mouse cells or the like and microorganisms such as *E. coli*, *Bacillus subtilis*, yeast or the like.

[0023] The invention relates to a process for producing the modified antibodies, which comprises culturing the above-mentioned hosts and extracting the modified antibodies from the culture thereof.

[0024] The present invention further relates to a process for producing a dimer of the single chain Fv which comprises culturing host animal cells producing the single chain Fv in a serum-free medium to secrete the single chain Fv into the medium and isolating the dimer of the single chain Fv formed in the medium.

[0025] The present invention also relates to the use of the modified antibodies as an agonist. That is, it relates to the signal-transduction agonist which comprises as an active ingredient the modified antibody obtained as mentioned above. Since the modified antibodies used in the invention are those that crosslink the receptor on the cell surface and induce signal transduction, the receptor can be any receptor that is oligomerized, e.g. dimerized, by combining with the ligand and thereby transduce a signal into cells. The receptor includes hormone receptors and cytokine receptors. The hormone receptor includes, for example, estrogen receptor. The cytokine receptor and the like include hematopoietic factor receptor, lymphokine receptor, growth factor receptor,

differentiation control factor receptor and the like. Examples of cytokine receptors are erythropoietin (EPO) receptor, thrombopoietin (TPO) receptor, granulocyte colony stimulating factor (G-CSF) receptor, macrophage colony stimulating factor (M-CSF) receptor, granular macrophage colony stimulating factor (GM-CSF) receptor, tumor necrosis factor (TNF) receptor, interleukin-1 (IL-1) receptor, interleukin-2 (IL-2) receptor, interleukin-3 (IL-3) receptor, interleukin-4 (IL-4) receptor, interleukin-5 (IL-5) receptor, interleukin-6 (IL-6) receptor, interleukin-7 (IL-7) receptor, interleukin-9 (IL-9) receptor, interleukin-10 (IL-10) receptor, interleukin-11 (IL-11) receptor, interleukin-12 (IL-12) receptor, interleukin-13 (IL-13) receptor, interleukin-15 (IL-15) receptor, interferon-alpha (IFN-alpha) receptor, interferon-beta (IFN-beta) receptor, interferon-gamma (IFN-gamma) receptor, growth hormone (GH) receptor, insulin receptor, blood stem cell proliferation factor (SCF) receptor, vascular epidermal growth factor (VEGF) receptor, epidermal cell growth factor (EGF) receptor, nerve growth factor (NGF) receptor, fibroblast growth factor (FGF) receptor, platelet-derived growth factor (PDGF) receptor, transforming growth factor-beta (TGF-beta) receptor, leukocyte migration inhibitory factor (LIF) receptor, ciliary neurotrophic factor (CNTF) receptor, oncostatin M (OSM) receptor, Notch family receptor and the like. Therefore, the pharmaceutical preparations containing the agonist modified antibody as an active ingredient are useful for as, for example, preventives and/or remedies for various disease such as cancers, inflammation, hormone disorders and blood diseases.

[0026] The modified antibodies of the present invention comprise two or more H chain V regions and two or more L chain V regions derived from monoclonal antibodies. The structure of the modified antibodies may be a dimer of single chain Fv comprising one H chain V region and one L chain V region or a polypeptide comprising two H chain V regions and two L chain V regions. In the modified antibodies of the invention, the V regions of H chain and L chain are preferably linked through a peptide linker which consists of one or more amino acids. The resulting modified antibodies contain variable regions of the parent antibodies and retain the complementarity determining region (CDR) thereof, and therefore bind to the antigen with the same specificity as that of the parent monoclonal antibodies.

[0027] H Chain V Region

[0028] In the present invention, the H chain V region derived from a monoclonal antibody recognizes a cell surface molecule(s), for example, a protein (a receptor or a protein involved in signal transduction) or a sugar chain of the protein or on cell membrane and oligomerizes, for example, dimerizes through crosslinking of said molecule, and thereby serves as an agonist transducing a signal into the cells. The H chain V region of the invention includes H chain V regions derived from a mammal (e.g. human, mouse, rat, bovine, sheep, ape etc.) and partially modified H chain V regions thereof. More preferable is a humanized H chain V region containing FR of H chain V region of a human monoclonal antibody and CDR of H chain V region of a mouse monoclonal antibody. The H chain V region further can be an H chain V region derived from a human monoclonal antibody corresponding to the aforementioned H chain V region of mouse monoclonal antibody, which can be produced by recombination technique. The H chain V region

of the invention may be a fragment of aforementioned H chain V region, which fragment preserves the antigen binding capacity.

[0029] L Chain V Region

[0030] In the present invention, the L chain V region derived from the monoclonal antibody recognizes a cell surface molecule(s), for example, a protein (a receptor or a protein involved in signal transduction) or a sugar chain of the protein or on cell membrane and oligomerizes, for example, dimerizes through crosslinking of said molecule, and thereby serves as an agonist transducing a signal into the cells. The L chain V region of the invention includes L chain V regions derived from a mammal (e.g. human, mouse, rat, bovine, sheep, ape etc.) and partially modified L chain V regions thereof. More preferable is a humanized L chain V region containing FR of L chain V region of human monoclonal antibody and CDR of L chain V region of mouse monoclonal antibodies. The L chain V regions further can be an L chain V region derived from human monoclonal antibody corresponding to the aforementioned L chain V region of mouse monoclonal antibody, which can be produced by recombination technique. The L chain V regions of the invention may be fragments of L chain V region, which fragments preserve the antigen binding capacity.

[0031] Complementarity Determining Region (CDR)

[0032] Each V region of L chain and H chain forms an antigen-binding site. The variable region of the L and H chains is composed of comparatively conserved four common framework regions linked to three hypervariable regions or complementarity determining regions (CDR) (Kabat, E. A. et al., "Sequences of Protein of Immunological Interest", US Dept. Health and Human Services, 1983).

[0033] Major portions in the four framework regions (FRs) form β -sheet structures and thus three CDRs form a loop. CDRs may form a part of the β -sheet structure in certain cases. The three CDRs are held sterically close position to each other by FR, which contributes to the formation of the antigen-binding site together with three CDRs.

[0034] These CDRs can be identified by comparing the amino acid sequence of V region of the obtained antibody with known amino acid sequences of v regions of known antibodies according to the empirical rule in Kabat, E. A. et al., "Sequences of Protein of Immunological Interest".

[0035] Single Chain Fv

[0036] A single chain Fv is a polypeptide monomer comprising an H chain V region and an L chain V region linked each other which are derived from monoclonal antibodies. The resulting single chain Fvs contain variable regions of the parent monoclonal antibodies and preserve the complementarity determining region thereof, and therefore the single chain Fvs bind to the antigen by the same specificity as that of the parent monoclonal antibodies (JP-Appl. 11-63557). A part of the variable region and/or CDR of the single chain Fv of the invention or a part of the amino acid sequence thereof may be partially altered, for example deleted, replaced or added. The H chain V region and L chain V region composing the single chain Fv of the invention are mentioned before and may be linked directly or through a linker, preferably a peptide linker. The consti-

tution of the single chain Fv may be [H chain V region]-[L chain V region] or [L chain V region]-[H chain V region]. In the present invention, it is possible to make the single chain Fv to form a dimer, a trimer or a tetramer, from which the modified antibody of the invention can be formed.

[0037] Single Chain Modified Antibody

[0038] The single chain modified antibodies of the present invention comprising two or more H chain V regions and two or more L chain V regions, preferably each two to four, especially preferable each two comprise two or more H chain V regions and L chain V regions as mentioned above. Each region of the peptide should be arranged such that the modified single chain antibody forms a specific steric structure, concretely mimicking a steric structure formed by the dimer of single chain Fv. For instance, the V regions are arranged in the order of the following manner:

[0039] [H chain V region]-[L chain V region]-[H chain V region]-[L chain V region]; or

[0040] [L chain V region]-[H chain V region]-[L chain V region]-[H chain V region],

[0041] wherein these regions are connected through a peptide linker, respectively.

[0042] Linker

[0043] In this invention, the linkers for the connection between the H chain V region and the L chain V region may be any peptide linker which can be introduced by the genetic engineering procedure or any linker chemically synthesized. For instance, linkers disclosed in literatures, e.g. Protein Engineering, 9(3), 299-305, 1996 may be used in the invention. These linkers can be the same or different in the same molecule. If peptide linkers are required, the following are cited as example linkers:

[0044] Ser

[0045] Gly-Ser

[0046] Gly-Gly-Ser

[0047] Ser-Gly-Gly

[0048] Gly-Gly-Gly-Ser

[0049] Ser-Gly-Gly-Gly

[0050] Gly-Gly-Gly-Gly-Ser

[0051] Ser-Gly-Gly-Gly-Gly

[0052] Gly-Gly-Gly-Gly-Gly-Ser

[0053] Ser-Gly-Gly-Gly-Gly-Gly

[0054] Gly-Gly-Gly-Gly-Gly-Gly-Ser

[0055] Ser-Gly-Gly-Gly-Gly-Gly-Gly

[0056] (Gly-Gly-Gly-Gly-Ser)_n and

[0057] (Ser-Gly-Gly-Gly-Gly)_n

[0058] wherein n is an integer not less than one. Preferable length of the linker peptide varies dependent upon the receptor to be the antigen, in the case of single chain Fvs, the range of 1 to 20 amino acids is normally preferable. In the case of single chain modified antibodies comprising two or more H chain V regions and two or more L chain V regions, the peptide linkers connecting those forming the same

antigen binding site comprising [H chain V region]-[L chain V region] (or [L chain V region]-[H chain V region]) have lengths of 1-30 amino acids, preferably 1-20 amino acids, more preferably 3-18 amino acids. The peptide linkers connecting those not forming the same antigen binding site comprising [H chain V region]-[L chain V region] or ([L chain V region]-[H chain V region]) have lengths of 1-40 amino acids, preferably 3-30 amino acids, more preferably 5-20 amino acids. The method for introducing those linkers will be described in the explanation for DNA construction coding for modified antibodies of the invention.

[0059] The chemically synthesized linkers, i.e. the chemical crosslinking agents, according to the invention can be any linkers conventionally employed for the linkage of peptides. Examples of the linkers may include N-hydroxy succinimide (NHS), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS³), dithiobis(succinimidyl propionate) (DSP), dithiobis(sulfosuccinimidyl propionate) (DTSSP), ethylene glycolbis(succinimidyl succinate) (EGS), ethylene glycolbis(sulfosuccinimidyl succinate) (sulfo-EGS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo-DST), bis[2-(succinimido oxycarbonyloxy)ethyl]sulfone (BSOCOES), bis[2-(sulfosuccinimido oxycarbonyloxy)ethyl]sulfone. (sulfo-BSOCOES) or the like. These are commercially available.

[0060] To form a dimer of the single chain Fv it is preferable to select a linker suitable to dimerize in the solution such as culture medium more than 20%, preferably more than 50%, more preferably more than 80%, most preferably more than 90% of the single chain Fv produced in the host cells. Specifically, preferable is a linker composed of 2 to 12 amino acids, preferably 3 to 10 amino acids or other linkers corresponding thereto.

[0061] Preparation of Modified Antibodies

[0062] The modified antibodies can be produced by connecting, through the aforementioned linker, an H chain V region and an L chain V region derived from known or novel monoclonal antibodies specifically binding to a cell surface molecule(s). As examples of the single chain Fvs are cited MABL1-scFv and MABL2-scFv comprising the H chain V region and the L chain V region derived from the antibody MABL-1 and the antibody MABL-2, respectively. As examples of the single chain polypeptides comprising two H chain V regions and two L chain V regions are cited MABL1-sc(Fv)₂ and MABL2-sc(Fv)₂ comprising the H chain V region and the L chain V region derived from the aforementioned antibodies.

[0063] For the preparation of the polypeptide, a signal peptide may be attached to N-terminal of the polypeptide if the polypeptide is desired to be a secretory peptide. A well-known amino acid sequence useful for the purification of polypeptide such as the FLAG sequence may be attached for the efficient purification of the polypeptide. In this case a dimer can be formed by using anti-FLAG antibody.

[0064] For the preparation of the modified antibody of the invention, it is necessary to obtain a DNA, i.e. a DNA encoding the single chain Fv or a DNA encoding reconstructed single chain polypeptide. These DNAs, especially for MABL1-scFv, MABL2-scFv, MABL1-sc(Fv)₂ and/or MABL2-sc(Fv)₂ are obtainable from the DNAs encoding the H chain V region and the L chain V region derived from said

Fv. They are also obtainable by PCR method using those DNA as a template and amplifying the part of DNA contained therein encoding desired amino acid sequence with the aid of a pair of primers corresponding to both ends thereof.

[0065] In the case where each V region having partially modified amino acid sequence is desired, the V regions in which one or some amino acids are modified, i.e. deleted, replaced or added can be obtained by a procedure known in the art using PCR. A part of the amino acid sequence in the V region is preferably modified by the PCR known in the art in order to prepare the modified antibody which is sufficiently active against the specific antigen.

[0066] For the determination of primers for the PCR amplification, it is necessary to decide the type of the H chain and L chain of the desired antibodies. In the case of antibody MABL-1 and the antibody MABL-2 it has been reported, however, that the antibody MABL-1 has κ type L chains and γ 1 type H chains and the antibody MABL-2 has κ type L chains and γ 2a type H chains (JP-Appl. 11-63557). For the PCR amplification of the DNA encoding the H chain and L chain of the antibody MABL-1 and/or the antibody MABL-2, primers described in Jones, S. T. et al., *Bio/Technology*, 9, 88-89, 1991 may be employed.

[0067] For the amplification of the L chain V regions of the antibody MABL-1 and the antibody MABL-2 using the polymerase chain reaction (PCR), 5'-end and 3'-end oligonucleotide primers are decided as aforementioned. In the same manner, 5'-end and 3'-end oligonucleotide primers are decided for the amplification of the H chain V regions of the antibody MABL-1 and the antibody MABL-2.

[0068] In embodiments of the invention, the 5'-end primers which contain a sequence "GATC" providing the restriction enzyme *Hinf* I recognition site at the neighborhood of 5'-terminal thereof are used and the 3'-end primers which contain a nucleotide sequence "CCCGGG" providing the *Xma*I recognition site at the neighborhood of 5'-terminal thereof are used. Other restriction enzyme recognition site may be used instead of these sites as long as they are used for subcloning a desired DNA fragment into a cloning vector.

[0069] Specifically designed PCR primers are employed to provide suitable nucleotide sequences at 5'-end and 3'-end of the cDNAs encoding the V regions of the antibodies MABL-1 and MABL-2 so that the cDNAs are readily inserted into an expression vector and appropriately function in the expression vector (e.g. this invention devises to increase transcription efficiency by inserting Kozak sequence). The V regions of the antibodies MABL-1 and MABL-2 obtained by amplifying by PCR using these primers are inserted into HEF expression vector containing the desired human C region (see WO92/19759). The cloned DNAs can be sequenced by using any conventional process which comprises, for example, inserting the DNAs into a suitable vector and then sequencing using the automatic DNA sequencer (Applied Biosystems).

[0070] A linker such as a peptide linker can be introduced into the modified antibody of the invention in the following manner. Primers which have partially complementary sequence with the primers for the H chain V regions and the L chain V regions as described above and which code for the

N-terminal or the C-terminal of the linker are designed. Then, the PCR procedure can be carried out using these primers to prepare a DNA encoding the peptide linker having desired amino acid sequence and length. The DNAs encoding the H chain V region and the L chain V region can be connected through the resulting DNA to produce the DNA encoding the modified antibody of the invention which has the desired peptide linker. Once the DNA encoding one of the modified antibodies is prepared, the DNAs encoding the modified antibodies with or without the desired peptide linker can readily be produced by designing various primers for the linker and then carrying out the PCR using the primers and the aforementioned DNA as a template.

[0071] Each V region of the modified antibody of the present invention can be humanized by using conventional techniques (e.g. Sato, K. et al., *Cancer Res.*, 53, 1-6 (1993)). Once a DNA encoding a humanized Fv is prepared, a humanized single chain Fv, a fragment of the humanized single chain Fv, a humanized monoclonal antibody and a fragment of the humanized monoclonal antibody can readily be produced according to conventional methods. Preferably, amino acid sequences of the V regions thereof may be partially modified, if necessary.

[0072] Furthermore, a DNA derived from other mammalian origin, for example a DNA of human, can be produced in the same manner as used to produce DNA encoding the H chain V region and the L chain V region derived from mouse mentioned in the above. The resulting DNA can be used to prepare an H chain V region and an L chain V region of other mammal, especially human origin, a single chain Fv derived from human and a fragment thereof, and a monoclonal antibody of human origin and a fragment thereof.

[0073] As mentioned above, when the aimed DNAs encoding the V regions of the modified antibodies and the V regions of the humanized modified antibodies are prepared, the expression vectors containing them and hosts transformed with the vectors can be obtained according to conventional methods. Further, the hosts can be cultured according to a conventional method to produce the reconstructed single chain Fv, the reconstructed humanized single chain Fv, the humanized monoclonal antibodies and fragments thereof. They can be isolated from cells or a medium and can be purified into a homogeneous mass. For this purpose any isolation and purification methods conventionally used for proteins, e.g. chromatography, ultra-filtration, salting-out and dialysis, may be employed in combination, if necessary, without limitation thereto.

[0074] When the reconstructed single chain Fv of the present invention is produced by culturing an animal cell such as COS7 cells or CHO cells, preferably CHO cells, in a serum-free medium, the reconstructed single chain Fv is efficiently dimerized in the medium. The dimer of the single chain Fv as formed above can be isolated stably and efficiently and preserved for a long period in the dimer form. The serum-free medium employed in the invention may be any medium conventionally used for the production of a recombinant protein without limit thereto.

[0075] For the production of the modified antibodies of the present invention, any expression systems can be employed, for example, eukaryotic cells such as animal cells, e.g., established mammalian cell lines, filamentous fungi and yeast, and prokaryotic cells such as bacterial cells e.g., *E.*

coli. Preferably, the modified antibodies of the invention are expressed in mammalian cells, for example COS7 cells or CHO cells.

[0076] For the production of the reconstructed polypeptides binding to cells with human IAP of the present invention, any expression systems can be employed, for example, eukaryotic cells such as animal cells, e.g., established mammalian cell lines, filamentous fungi and yeast, and prokaryotic cells such as bacterial cells e.g., *E. coli*. Preferably, the reconstructed polypeptides of the invention are expressed in mammalian cells, for example COS7 cells or CHO cells.

[0077] In these cases, conventional promoters useful for the expression in mammalian cells can be used. Preferably, human cytomegalovirus (HCMV) immediate early promoter is used. Expression vectors containing the HCMV promoter include HCMV-VH-HC γ 1, HCMV-VL-HCK and the like which are derived from pSV2neo (WO92/19759).

[0078] Additionally, other promoters for gene expression in mammal cell which may be used in the invention include virus promoters derived from retrovirus, polyoma virus, adenovirus and simian virus 40 (SV40) and promoters derived from mammal such as human polypeptide-chain elongation factor-1 α (HEF-1 α). SV40 promoter can easily be used according to the method of Mulligan, R. C., et al. (Nature 277, 108-114 (1979)) and HEF-1 α promoter can also be used according to the methods of Mizushima, S. et al. (Nucleic Acids Research, 18, 5322 (1990)).

[0079] Replication origin (ori) which can be used in the invention includes ori derived from SV40, polyoma virus, adenovirus, bovine papilloma virus (BPV) and the like. An expression vector may contain, as a selection marker, phosphotransferase APH (3') II or I (neo) gene, thymidine kinase (TK) gene, *E. coli* xanthine-guanine phosphoribosyl transferase (Ecogpt) gene or dihydrofolate reductase (DHFR) gene.

[0080] The antigen-binding activity of the modified antibody as prepared above can be evaluated using the binding-inhibitory ability of original antibodies as an index. Concretely, the activity is evaluated in terms of the absence or presence of concentration-dependent inhibition of the binding of said monoclonal antibody as an index.

[0081] More in detail, animal cells transformed with an expression vector containing a DNA encoding the modified antibody of the invention, e.g., COS7 cells or CHO cells, are cultured. The cultured cells and/or the supernatant of the medium or the modified antibody purified from them are used to determine the binding to antigen. As a control is used a supernatant of the culture medium in which cells transformed only with the expression vector were cultured. In the case of an antigen, for example, the antibody MABL-1 and the antibody MABL-2, a test sample of the modified antibody of the invention or the supernatant of the control is added to mouse leukemia cell line, L1210 cells, expressing human IAP and then an assay such as the flow cytometry is carried out to evaluate the antigen-binding activity.

[0082] In vitro evaluation of the signal transduction effect (apoptosis-inducing effect in the cases of the antibody MABL-1 and the antibody MABL-2) is performed in the following manner: A test sample of the above modified antibody is added to the cells which are expressing the antibody or cells into which the gene for the antibody has

been introduced, and is evaluated by the change caused by the signal transduction, for example, whether cell death is induced in a manner specific to the human IAP-antigen.

[0083] In vivo evaluation of the apoptosis-inducing effect, for example, in the case where the modified antibody recognizes human IAP (e.g. modified antibodies derived from the antibody MABL-1 and the antibody MABL-2) is carried out in the following manner: A mouse model of human myeloma is prepared. To the mice is intravenously administered the monoclonal antibody or the modified antibody of the invention, which induces apoptosis of nucleated blood cells having IAP. To mice of a control group is administered PBS alone. The induction of apoptosis is evaluated in terms of antitumor effect based on the change of human IgG content in serum of the mice and their survival time.

[0084] The modified antibodies of the invention, which comprises two or more H chain V regions and two or more L chain V regions, preferably each two to four, more preferably each two, may be a dimer of the single chain Fv comprising one H chain V region and one L chain V region, or a single chain polypeptide in which two or more H chain V regions and two or more L chain V regions are connected. It is considered that owing to such construction the peptide mimics three dimensional structure of the antigen binding site of the parent monoclonal antibody and therefore retains an excellent antigen-binding property.

[0085] The modified antibodies of the invention has been remarkably lowered in the molecular size compared with antibody molecule (whole IgG), and, therefore, have superior permeability into tissues and tumors and higher activity than original monoclonal antibodies. Therefore, it is possible to transduce various signals into cells by properly selecting the original antibody which is modified. The pharmaceutical preparations containing them are useful for treating diseases curable by inducing signal transduction, for example cancers, inflammation, hormone disorders as well as blood dyscrasia, for example, leukemia, malignant lymphoma, aplastic anemia, myelodysplasia syndrome and polycythemia vera. It is further expected that the antibody of the invention can be used as a contrast agent by RI-labeling. The effect can be enhanced by attaching to a RI-compound or a toxin.

[0086] The present invention is illustrated by examples, which by no means restrict the scope of the invention, using monoclonal antibodies binding to human IAP (the antibody MABL-1 and the antibody MABL-2).

BEST MODE FOR WORKING THE INVENTION

[0087] The present invention will concretely be illustrated in reference to the following examples, which in no way limit the scope of the invention.

[0088] For illustrating the production process of the modified antibodies of the invention, examples of producing single chain Fvs are shown below. Mouse antibodies against human IAP, MABL-1 and MABL-2 were used in the examples of producing the modified antibodies. Hybridomas MABL-1 and MABL-2 producing them respectively were internationally deposited as FERM BP-6100 and FERM BP-6101 with the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Tech-

nology, Minister of International Trade and Industry (1-3 Higasi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan), an authorized depository for microorganisms, on Sep. 11, 1997.

EXAMPLE 1

Cloning of DNAs Encoding V Region of Mouse Monoclonal Antibodies to Human IAP

[0089] DNAs encoding variable regions of the mouse monoclonal antibodies to human IAP, MABL-1 and MABL-2, were cloned as follows.

[0090] 1.1 Preparation of Messenger RNA (mRNA)

[0091] mRNAs of the hybridomas MABL-1 and MABL-2 were obtained by using mRNA Purification Kit (Pharmacia Biotech).

[0092] 1.2 Synthesis of Double-Stranded cDNA

[0093] Double-stranded cDNA was synthesized from about 1 μ g of the mRNA using Marathon cDNA Amplification Kit (CLONTECH) and an adapter was linked thereto.

[0094] 1.3 PCR Amplification of Genes Encoding Variable Regions of an Antibody by

[0095] PCR was carried out using Thermal Cycler (PERKIN ELMER).

[0096] (1) Amplification of a Gene Coding for L Chain V Region of MABL-1

[0097] Primers used for the PCR method are Adapter Primer-1 (CLONTECH) shown in SEQ ID No. 1, which hybridizes to a partial sequence of the adapter, and MKC (Mouse Kappa Constant) primer (Bio/Technology, 9, 88-89, 1991) shown in SEQ ID No. 2, which hybridizes to the mouse kappa type L chain V region.

[0098] 50 μ l of the PCR solution contains 5 μ l of 10 \times PCR Buffer II, 2 mM MgCl₂, 0.16 mM dNTPs (dATP, dGTP, dCTP and dTTP), 2.5 units of a DNA polymerase, AmpliTaq Gold (PERKIN ELMER), 0.2 μ M of the adapter primer of SEQ ID No. 1, 0.2 μ M of the MKC primer of SEQ ID No. 2 and 0.1 μ g of the double-stranded cDNA derived from MABL-1. The solution was preheated at 94° C. of the initial temperature for 9 minutes and then heated at 94° C. for 1 minute, at 60° C. for 1 minute and at 72° C. for 1 minute 20 seconds in order. This temperature cycle was repeated 35 times and then the reaction mixture was further heated at 72° C. for 10 minutes.

[0099] (2) Amplification of cDNA Encoding H Chain V Region of MABL-1

[0100] The Adapter Primer-1 shown in SEQ ID No. 1 and MHC- γ 1 (Mouse Heavy Constant) primer (Bio/Technology, 9, 88-89, 1991) shown in SEQ ID No. 3 were used as primers for PCR.

[0101] The amplification of cDNA was performed according to the method of the amplification of the L chain V region gene, which was described in Example 1.3-(1), except for using 0.2 μ M of the MHC- γ 1 primer instead of 0.2 μ M of the MKC primer.

[0102] (3) Amplification of cDNA Encoding L Chain V Region of MABL-2

[0103] The Adapter Primer-1 of SEQ ID No. 1 and the MKC primer of SEQ ID No. 2 were used as primers for PCR.

[0104] The amplification of cDNA was carried out according to the method of the amplification of the L chain V region gene of MABL-1 which was described in Example 1.3-(1), except for using 0.1 μ g of the double-stranded cDNA derived from MABL-2 instead of 0.1 μ g of the double-stranded cDNA from MABL-1.

[0105] (4) Amplification of cDNA Encoding H Chain V Region of MABL-2

[0106] The Adapter Primer-1 of SEQ ID No. 1 and MHC- γ 2a primer (Bio/Technology, 9, 88-89, 1991) shown in SEQ ID No. 4 were used as primers for PCR.

[0107] The amplification of cDNA was performed according to the method of the amplification of the L chain V region gene, which was described in Example 1.3-(3), except for using 0.2 μ M of the MHC- γ 2a primer instead of 0.2 μ M of the MKC primer.

[0108] 1.4 Purification of PCR Products

[0109] The DNA fragment amplified by PCR as described above was purified using the QIAquick PCR Purification Kit (QIAGEN) and dissolved in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA.

[0110] 1.5 Ligation and Transformation

[0111] About 140 ng of the DNA fragment comprising the gene encoding the mouse kappa type L chain V region derived from MABL-1 as prepared above was ligated with 50 ng of pGEM-T Easy vector (Promega) in the reaction buffer comprising 30 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP and 3 units of T4 DNA Ligase (Promega) at 15° C. for 3 hours.

[0112] Then, 1 μ l of the reaction mixture was added to 50 μ l of *E. coli* DH5 α competent cells (Toyobo Inc.) and the cells were stored on ice for 30 minutes, incubated at 42° C. for 1 minute and stored on ice for 2 minutes again. 100 μ l of SOC medium (GIBCO BRL) was added. The cells of *E. coli* were plated on LB (Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Laboratory Press, 1989) agar medium containing 100 μ g/ml of ampicillin (SIGMA) and cultured at 37° C. overnight to obtain the transformant of *E. coli*.

[0113] The transformant was cultured in 3 ml of LB medium containing 50 μ g/ml of ampicillin at 37° C. overnight and the plasmid DNA was prepared from the culture using the QIAprep Spin Miniprep Kit (QIAGEN).

[0114] The resulting plasmid comprising the gene encoding the mouse kappa type L chain V region derived from the hybridoma MABL-1 was designated as pGEM-M1L.

[0115] According to the same manner as described above, a plasmid comprising the gene encoding the mouse H chain V region derived from the hybridoma MABL-1 was prepared from the purified DNA fragment and designated as pGEM-M1H.

[0116] A plasmid comprising the gene encoding the mouse kappa type L chain V region derived from the hybridoma MABL-2 was prepared from the purified DNA fragment and designated as pGEM-M2L.

[0117] A plasmid comprising the gene encoding the mouse H chain V region derived from the hybridoma MABL-2 was prepared from the purified DNA fragment and designated as pGEM-M2H.

EXAMPLE 2

DNA Sequencing

[0118] The nucleotide sequence of the cDNA encoding region in the aforementioned plasmids was determined using Auto DNA Sequencer (Applied Biosystem) and ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem) according to the manufacturer's protocol.

[0119] The nucleotide sequence of the gene encoding the L chain V region from the mouse antibody MABL-1, which is included in the plasmid pGEM-M1L, is shown in SEQ ID No. 5.

[0120] The nucleotide sequence of the gene encoding the H chain V region from the mouse antibody MABL-1, which is included in the plasmid pGEM-M1H, is shown in SEQ ID No. 6.

[0121] The nucleotide sequence of the gene encoding the L chain V region from the mouse antibody MABL-2, which is included in the plasmid pGEM-M2L, is shown in SEQ ID No. 7.

[0122] The nucleotide sequence of the gene encoding the H chain V region from the mouse antibody MABL-2, which is included in the plasmid pGEM-M2H, is shown in SEQ ID No. 8.

EXAMPLE 3

Determination of CDR

[0123] The V regions of L chain and H chain generally have a similarity in their structures and each four framework regions therein are linked by three hypervariable regions, i.e., complementarity determining regions (CDR). An amino acid sequence of the framework is relatively well conserved, while an amino acid sequence of CDR has extremely high-variation (Kabat, E. A., et al., "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services, 1983).

[0124] On the basis of these facts, the amino acid sequences of the variable regions from the mouse monoclonal antibodies to human IAP were applied to the database of amino acid sequences of the antibodies made by Kabat et al. to investigate the homology. The CDR regions were determined based on the homology as shown in Table 1.

TABLE 1

Plasmid	SEQ ID No.	CDR(1)	CDR(2)	CDR(3)
pGEM-M1L	5	43-58	74-80	113-121
pGEM-M1H	6	50-54	69-85	118-125
pGEM-M2L	7	43-58	74-80	113-121
pGEM-M2H	8	50-54	69-85	118-125

EXAMPLE 4

Identification of Cloned cDNA Expression

Preparation of Chimera MABL-1 Antibody and Chimera MABL-2 Antibody

[0125] 4.1 Preparation of Vectors Expressing Chimera MABL-1 Antibody

[0126] cDNA clones, pGEM-M1L and pGEM-M1H, encoding the V regions of the L chain and the H chain of the mouse antibody MABL-1, respectively, were modified by the PCR method and introduced into the HEF expression vector (WO92/19759) to prepare vectors expressing chimera MABL-1 antibody.

[0127] A forward primer MLS-(SEQ ID No. 9) for the L chain V region and a forward primer MHS (SEQ ID No. 10) for the H chain V region were designed to hybridize to a DNA encoding the beginning of the leader sequence of each V region and to contain the Kozak consensus sequence (J. Mol. Biol., 196, 947-950, 1987) and HindIII restriction enzyme site. A reverse primer MLAS (SEQ ID No. 11) for the L chain V region and a reverse primer MHAS (SEQ ID No. 12) for the H chain V region were designed to hybridize to a DNA encoding the end of the J region and to contain the splice donor sequence and BamHI restriction enzyme site.

[0128] 100 μ l of a PCR solution comprising 10 μ l of 10 \times PCR Buffer II, 2 mM MgCl₂, 0.16 mM dNTPs (dATP, dGTP, dCTP and dTTP), 5 units of DNA polymerase AmpliTaq Gold, 0.4 μ M each of primers and 8 ng of the template DNA (pGEM-M1L or pGEM-M1H) was preheated at 94° C. of the initial temperature for 9 minutes and then heated at 94° C. for 1 minute, at 60° C. for 1 minute and at 72° C. for 1 minute 20 seconds in order. This temperature cycle was repeated 35 times and then the reaction mixture was further heated at 72° C. for 10 minutes.

[0129] The PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN) and then digested with HindIII and BamHI. The product from the L chain V region was cloned into the HEF expression vector, HEF- κ and the product from the H chain V region was cloned into the HEF expression vector, HEF- γ . After DNA sequencing, plasmids containing a DNA fragment with a correct DNA sequence are designated as HEF-M1L and HEF-M1H, respectively.

[0130] 4.2 Preparation of Vectors Expressing Chimera MABL-2 Antibodies

[0131] Modification and cloning of cDNA were performed in the same manner described in Example 4.1 except for using pGEM-M2L and pGEM-M2H as template DNA instead of pGEM-M1L and pGEM-M1H. After DNA sequencing, plasmids containing DNA fragments with correct DNA sequences are designated as HEF-M2L and HEF-M2H, respectively.

[0132] 4.3 Transfection to COS7 Cells

[0133] The aforementioned expression vectors were tested in COS7 cells to observe the transient expression of the chimera MABL-1 and MABL-2 antibodies.

[0134] (1) Transfection with Genes for the Chimera MABL-1 Antibody

[0135] COS7 cells were co-transformed with the HEF-M1L and HEF-M1H vectors by electroporation using the Gene Pulser apparatus (BioRad). Each DNA (10 μ g) and 0.8 ml of PBS with 1×10^7 cells/ml were added to a cuvette. The mixture was treated with pulse at 1.5 kV, 25 μ F of electric capacity.

[0136] After the restoration for 10 minutes at a room temperature, the electroporated cells were transferred into DMEM culture medium (GIBCO BRL) containing 10% γ -globulin-free fetal bovine serum. After culturing for 72 hours, the supernatant was collected, centrifuged to remove cell fragments and recovered.

[0137] (2) Transfection with Genes Coding for the Chimera MABL-2 Antibody

[0138] The co-transfection to COS7 cells with the genes coding for the chimera MABL-2 antibody was carried out in the same manner as described in Example 4.3-(1) except for using the HEF-M2L and HEF-M2H vectors instead of the HEF-M1L and HEF-M1H vectors. The supernatant was recovered in the same manner.

[0139] 4.4 Flow Cytometry

[0140] Flow cytometry was performed using the aforementioned culture supernatant of COS7 cells to measure binding to the antigen. The culture supernatant of the COS7 cells expressing the chimera MABL-1 antibody or the COS7 cells expressing the chimera MABL-2 antibody, or human IgG antibody (SIGMA) as a control was added to 4×10^5 cells of mouse leukemia cell line L1210 expressing human IAP and incubated on ice. After washing, the FITC-labeled anti-human IgG antibody (Cappel) was added thereto. After incubating and washing, the fluorescence intensity thereof was measured using the FACScan apparatus (BECTON DICKINSON).

[0141] Since the chimera MABL-1 and MABL-2 antibodies were specifically bound to L1210 cells expressing human IAP, it is confirmed that these chimera antibodies have proper structures of the V regions of the mouse monoclonal antibodies MABL-1 and MABL-2, respectively (**FIGS. 1-3**).

EXAMPLE 5

Preparation of Reconstructed Single Chain Fv (scFv) of the Antibody MABL-1 and Antibody MABL-2

[0142] 5.1 Preparation of Reconstructed Single Chain Fv of Antibody MABL-1

[0143] The reconstructed single chain Fv of antibody MABL-1 was prepared as follows. The H chain V region and the L chain V of antibody MABL-1, and a linker were respectively amplified by the PCR method and were connected to produce the reconstructed single chain Fv of antibody MABL-1. The production method is illustrated in **FIG. 4**. Six primers (A-F) were employed for the production of the single chain Fv of antibody MABL-1. Primers A, C and E have a sense sequence and primers B, D and F have an antisense sequence.

[0144] The forward primer VHS for the H chain V region (Primer A, SEQ ID No. 13) was designed to hybridize to a DNA encoding the N-terminal of the H chain V region and

to contain NcoI restriction enzyme recognition site. The reverse primer VHAS for H chain V region (Primer B, SEQ ID No. 14) was designed to hybridize to a DNA coding the C-terminal of the H chain V region and to overlap with the linker.

[0145] The forward primer LS for the linker (Primer C, SEQ ID No. 15) was designed to hybridize to a DNA encoding the N-terminal of the linker and to overlap with a DNA encoding the C-terminal of the H chain V region. The reverse primer LAS for the linker (Primer D, SEQ ID No. 16) was designed to hybridize to a DNA encoding the C-terminal of the linker and to overlap with a DNA encoding the N-terminal of the L chain V region.

[0146] The forward primer VLS for the L chain V region (Primer E, SEQ ID No. 17) was designed to hybridize to a DNA encoding the C-terminal of the linker and to overlap with a DNA encoding the N-terminal of the L chain V region. The reverse primer VLAS-FLAG for L chain V region (Primer F, SEQ ID No. 18) was designed to hybridize to a DNA encoding the C-terminal of the L chain V region and to have a sequence encoding the FLAG peptide (Hopp, T. P. et al., *Bio/Technology*, 6, 1204-1210, 1988), two stop codons and EcoRI restriction enzyme recognition site.

[0147] In the first PCR step, three reactions, A-B, C-D and E-F, were carried out and PCR products thereof were purified. Three PCR products obtained from the first PCR step were assembled by their complementarity. Then, the primers A and F were added and the full length DNA encoding the reconstructed single chain Fv of antibody MABL-1 was amplified (Second PCR). In the first PCR, the plasmid pGEM-M1H encoding the H chain V region of antibody MABL-1 (see Example 2), a plasmid pSC-DP1 which comprises a DNA sequence encoding a linker region comprising: Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser (SEQ ID No. 19) (Huston, J. S., et al., *Proc. Natl. Acad. Sci. USA*, 85, 5879-5883, 1988) and the plasmid pGEM-M1L encoding the L chain V region of antibody MABL-1 (see Example 2) were employed as template, respectively.

[0148] 50 μ l of the solution for the first PCR step comprises 5 μ l of 10 \times PCR Buffer II, 2 mM MgCl₂, 0.16 mM dNTPs, 2.5 units of DNA polymerase, AmpliTaq Gold (PERKIN ELMER), 0.4 μ M each of primers and 5 ng each of template DNA. The PCR solution was preheated at 94° C. of the initial temperature for 9 minutes and then heated at 94° C. for 1 minute, at 65° C. for 1 minute and at 72° C. for 1 minute and 20 seconds in order. This temperature cycle was repeated 35 times and then the reaction mixture was further heated at 72° C. for 7 minutes.

[0149] The PCR products A-B (371 bp), C-D (63 bp) and E-F (384 bp) were purified using the QIAquick PCR Purification Kit (QIAGEN) and were assembled in the second PCR. In the second PCR, 98 μ l of a PCR solution comprising 120 ng of the first PCR product A-B, 20 ng of the PCR product C-D and 120 ng of the PCR product E-F, 10 μ l of 10 \times PCR Buffer II, 2 mM MgCl₂, 0.16 mM dNTPs, 5 units of DNA polymerase AmpliTaq Gold (PERKIN ELMER) was preheated at 94° C. of the initial temperature for 8 minutes and then heated at 94° C. for 2 minutes, at 65° C. for 2 minutes and at 72° C. for 2 minutes in order. This temperature cycle was repeated twice and then 0.4 μ M each of primers A and F were added into the reaction, respec-

tively. The mixture was preheated at 94° C. of the initial temperature for 1 minutes and then heated at 94° C. for 1 minute, at 65° C. for 1 minute and at 72° C. for 1 minute and 20 seconds in order. This temperature cycle was repeated 35 times and then the reaction mixture was further heated at 72° C. for 7 minutes.

[0150] A DNA fragment of 843 bp produced by the second PCR was purified and digested by NcoI and EcoRI. The resultant DNA fragment was cloned into pSCFVT7 vector. The expression vector pSCFVT7 contains a pelB signal sequence suitable for *E. coli* periplasmic expression system (Lei, S. P., et al., J. Bacteriology, 169, 4379-4383, 1987). After the DNA sequencing, the plasmid containing the DNA fragment encoding correct amino acid sequence of the reconstructed single chain Fv of antibody MABL-1 is designated as "pscM1" (see FIG. 5). The nucleotide sequence and the amino acid sequence of the reconstructed single chain Fv of antibody MABL-1 contained in the plasmid pscM1 are shown in SEQ ID No. 20.

[0151] The pscM1 vector was modified by the PCR method to prepare a vector expressing the reconstructed single chain Fv of antibody MABL-1 in mammalian cells. The resultant DNA fragment was introduced into pCHO1 expression vector. This expression vector, pCHO1, was constructed by digesting DHFR-ΔE-rvH-PM1-f (WO92/19759) with EcoRI and SmaI to eliminate the antibody gene and connecting the EcoRI-NotI-BamHI Adapter (Takara Shuzo) thereto.

[0152] As a forward primer for PCR, Sal-VHS primer shown in SEQ ID No. 21 was designed to hybridize to a DNA encoding the N-terminal of the H chain V region and to contain SalI restriction enzyme recognition site. As a reverse primer for PCR, FRH1anti primer shown in SEQ ID No. 22 was designed to hybridize to a DNA encoding the end of the first framework sequence.

[0153] 100 μl of PCR solution comprising 10 μl of 10×PCR Buffer II, 2 mM MgCl₂, 0.16 mM dNTPs, 5 units of the DNA polymerase, AmpliTaq Gold, 0.4 μM each of primer and 8 ng of the template DNA (pscM1) was preheated at 95° C. of the initial temperature for 9 minutes and then heated at 95° C. for 1 minute, at 60° C. for 1 minute and at 72° C. for 1 minute and 20 seconds in order. This temperature cycle was repeated 35 times and then the reaction mixture was further heated at 72° C. for 7 minutes.

[0154] The PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN) and digested by SalI and MboII to obtain a DNA fragment encoding the N-terminal of the reconstructed single chain Fv of antibody MABL-1. The pscM1 vector was digested by MboII and EcoRI to obtain a DNA fragment encoding the C-terminal of the reconstructed single chain Fv of antibody MABL-1. The SalI-MboII DNA fragment and the MboII-EcoRI DNA fragment were cloned into pCHO1-Igs vector. After DNA sequencing, the plasmid comprising the desired DNA sequence was designated as "pCHOM1" (see FIG. 6). The expression vector, pCHO1-Igs, contains a mouse IgG1 signal sequence suitable for the secretion-expression system in mammalian cells (Nature, 322, 323-327, 1988). The nucleotide sequence and the amino acid sequence of the reconstructed single chain Fv of antibody MABL-1 contained in the plasmid pCHOM1 are shown in SEQ ID No. 23.

[0155] 5.2 Preparation of Reconstructed Single Chain Fv of Antibody MABL-2

[0156] The reconstructed single chain Fv of antibody MABL-2 was prepared in accordance with the aforemen-

tioned Example 5.1. Employed in the first PCR step were plasmid pGEM-M2H encoding the H chain V region of MABL-2 (see Example 2) instead of pGEM-M1H and plasmid pGEM-M2L encoding the L chain V region of MABL-2 (see Example 2) instead of pGEM-M1L, to obtain a plasmid pscM2 which comprises a DNA fragment encoding the desired amino acid sequence of the single chain Fv of antibody MABL-2. The nucleotide sequence and the amino acid sequence of the reconstructed single chain Fv of antibody MABL-2 contained in the plasmid pscM2 are shown in SEQ ID No. 24.

[0157] The pscM2 vector was modified by the PCR method to prepare a vector, pCHOM2, for the expression in mammalian cells which contains the DNA fragment encoding the correct amino acid sequence of reconstructed single chain Fv of antibody MABL-2. The nucleotide sequence and the amino acid sequence of the reconstructed single chain Fv of antibody MABL-2 contained in the plasmid pCHOM2 are shown in SEQ ID No. 25.

[0158] 5.3 Transfection to COS7 cells

[0159] The pCHOM2 vector was tested in COS7 cells to observe the transient expression of the reconstructed single chain Fv of antibody MABL-2.

[0160] The COS7 cells were transformed with the pCHOM2 vector by electroporation using the Gene Pulser apparatus (BioRad). The DNA (10 μg) and 0.8 ml of PBS with 1×10⁷ cells/ml were added to a cuvette. The mixture was treated with pulse at 1.5 kV, 25 μF of electric capacity.

[0161] After the restoration for 10 minutes at a room temperature, the electroporated cells were transferred into IMDM culture medium (GIBCO BRL) containing 10% fetal bovine serum. After culturing for 72 hours, the supernatant was collected, centrifuged to remove cell fragments and recovered.

[0162] 5.4 Detection of the Reconstructed Single Chain Fv of Antibody MABL-2 in Culture Supernatant of COS7 Cells

[0163] The existence of the single chain Fv of antibody MABL-2 in the culture supernatant of COS7 cells which had been transfected with the pCHOM2 vector was confirmed by the Western Blotting method.

[0164] The culture supernatant of COS7 cells transfected with the pCHOM2 vector and the culture supernatant of COS7 cells transfected with the pCHO1 as a control were subjected to SDS electrophoresis and transferred to REINFORCED NC membrane (Schleicher & Schuell). The membrane was blocked with 5% skim milk (Morinaga Nyu-gyo), washed with 0.05% Tween 20-PBS and mixed with an anti-FLAG antibody (SIGMA). The membrane was incubated at room temperature, washed and mixed with alkaline phosphatase-conjugated mouse IgG antibody (Zymed). After incubating and washing at room temperature, the substrate solution (Kirkegaard Perry Laboratories) was added to develop color (FIG. 7).

[0165] A FLAG-peptide-specific protein was detected only in the culture supernatant of the pCHOM2 vector-introduced COS7 cells and thus it is confirmed that the reconstructed single chain Fv of antibody MABL-2 was secreted in this culture supernatant.

[0166] 5.5 Flow Cytometry

[0167] Flow cytometry was performed using the aforementioned COS7 cells culture supernatant to measure the binding to the antigen. The culture supernatant of the COS7 cells expressing the reconstructed single chain Fv of antibody MABL-2 or the culture supernatant of COS7 cells transformed with pCHO1 vector as a control was added to 2×10^5 cells of the mouse leukemia cell line L1210 expressing human Integrin Associated Protein (IAP) or the cell line L1210 transformed with pCOS1 as a control. After incubating on ice and washing, the mouse anti-FLAG antibody (SIGMA) was added. Then the cells were incubated and washed. Then, the FITC labeled anti-mouse IgG antibody (BECTON DICKINSON) was added thereto and the cells were incubated and washed again. Subsequently, the fluorescence intensity was measured using the FACScan apparatus (BECTON DICKINSON).

[0168] Since the single chain Fv of antibody MABL-2 was specifically bound to L1210 cells expressing human IAP, it is confirmed that the reconstructed single chain Fv of antibody MABL-2 has an affinity to human Integrin Associated Protein (IAP) (see **FIGS. 8-11**).

[0169] 5.6 Competitive ELISA

[0170] The binding activity of the reconstructed single chain Fv of antibody MABL-2 was measured based on the inhibiting activity against the binding of mouse monoclonal antibodies to the antigen.

[0171] The anti-FLAG antibody adjusted to $1 \mu\text{g/ml}$ was added to each well on 96-well plate and incubated at 37°C . for 2 hours. After washing, blocking was performed with 1% BSA-PBS. After incubating and washing at a room temperature, the culture supernatant of COS7 cells into which the secretion-type human IAP antigen gene (SEQ ID No. 26) had been introduced was diluted with PBS into twofold volume and added to each well. After incubating and washing at a room temperature, a mixture of $50 \mu\text{l}$ of the biotinized MABL-2 antibody adjusted to 100 ng/ml and $50 \mu\text{l}$ of sequentially diluted supernatant of the COS7 cells expressing the reconstructed single chain Fv of antibody MABL-2 were added into each well. After incubating and washing at a room temperature, the alkaline phosphatase-conjugated streptavidin (Zymed) was added into each well. After incubating and washing at a room temperature, the substrate solution (SIGMA) was added and absorbance of the reaction mixture in each well was measured at 405 nm.

[0172] The results revealed that the reconstructed single chain Fv of antibody MABL-2 (MABL2-scFv) evidently inhibited concentration-dependently the binding of the mouse antibody MABL-2 to human IAP antigen in comparison with the culture supernatant of the PCHO1-introduced COS7 cells as a control (**FIG. 12**). Accordingly, it is suggested that the reconstructed single chain Fv of antibody MABL-2 has the correct structure of each of the V regions from the mouse monoclonal antibody MABL-2.

[0173] 5.7 Apoptosis-Inducing Effect In Vitro

[0174] An apoptosis-inducing action of the reconstructed single chain Fv of antibody MABL-2 was examined by Annexin-V staining (Boehringer Mannheim) using the L1210 cells transfected with human IAP gene, the L1210 cells transfected with the pCOS1 vector as a control and CCRF-CEM cells.

[0175] To each 1×10^5 cells of the above cells was added the culture supernatant of the COS7 cells expressing the reconstructed single chain Fv of antibody MABL-2 or the culture supernatant of COS7 cells transfected with the pCHO1 vector as a control at 50% final concentration and the mixtures were cultured for 24 hours. Then, the Annexin-V staining was performed and the fluorescence intensity was measured using the FACScan apparatus (BECTON DICKINSON).

[0176] Results of the Annexin-V staining are shown in **FIGS. 13-18**, respectively. Dots in the left-lower region represent living cells and dots in the right-lower region represent cells at the early stage of apoptosis and dots in the right-upper region represent cells at the late stage of apoptosis. The results show that the reconstructed single chain Fv of antibody MABL-2 (MABL2-scFv) remarkably induced cell death of L1210 cells specific to human IAP antigen (**FIGS. 13-16**) and that the reconstructed single chain Fv also induced remarkable cell death of CCRF-CEM cells in comparison with the control (**FIGS. 17-18**).

[0177] 5.8 Expression of MABL-2 Derived Single Chain Fv in CHO Cells

[0178] CHO cells were transfected with the pCHOM2 vector to establish a CHO cell line which constantly expresses the single chain Fv (polypeptide) derived from the antibody MABL-2.

[0179] CHO cells were transformed with the pCHOM2 vector by the electroporation using the Gene Pulser apparatus (BioRad). A mixture of DNA ($10 \mu\text{g}$) and 0.7 ml of PBS with CHO cells (1×10^7 cells/ml) was added to a cuvette. The mixture was treated with pulse at 1.5 kv, $25 \mu\text{F}$ of electric capacity. After the restoration for 10 minutes at a room temperature, the electroporated cells were transferred into nucleic acid free α -MEM medium (GIBCO BRL) containing 10% fetal bovine serum and cultured. The expression of desired protein in the resultant clones was confirmed by SDS-PAGE and a clone with a high expression level was selected as a cell line producing the single chain Fv derived from the antibody MABL-2. The cell line was cultured in serum-free medium CHO-S-SFM II (GIBCO BRL) containing 10 nM methotrexate (SIGMA). Then, the culture supernatant was collected, centrifuged to remove cell fragments and recovered.

[0180] 5.9 Purification of MABL-2 Derived Single Chain Fv Produced in CHO Cells

[0181] The culture supernatant of the CHO cell line expressing the single chain Fv obtained in Example 5.8 was concentrated up to twenty times using a cartridge for the artificial dialysis (PAN130SF, ASAHI MEDICALS). The concentrated solution was stored at -20°C . and thawed on purification.

[0182] Purification of the single chain Fv from the culture supernatant of the CHO cells was performed using three kinds of chromatography, i.e., Blue-sepharose, a hydroxyapatite and a gel filtration.

[0183] (1) Blue-Sephacolumn Chromatography

[0184] The concentrated supernatant was diluted to ten times with 20 mM acetate buffer (pH 6.0) and centrifuged to remove insoluble materials ($10000 \times \text{rpm}$, 30 minutes). The supernatant was applied onto a Blue-sepharose column (20

ml) equilibrated with the same buffer. After washing the column with the same buffer, proteins adsorbed in the column were eluted by a stepwise gradient of NaCl in the same buffer, 0.1, 0.2, 0.3, 0.5 and up to 1.0 M. The pass-through fraction and each eluted fraction were analyzed by SDS-PAGE. The fractions in which the single chain Fv were confirmed (the fractions eluted at 0.1 to 0.3M NaCl) were pooled and concentrated up to approximately 20 times using CentriPrep-10 (AMICON).

[0185] (2) Hydroxyapatite

[0186] The concentrated solution obtained in (1) was diluted to 10 times with 10 mM phosphate buffer (pH 7.0) and applied onto the hydroxyapatite column (20 ml, BIO-RAD). The column was washed with 60 ml of 10 mM phosphate buffer (pH 7.0). Then, proteins adsorbed in the column were eluted by a linear gradient of sodium phosphate buffer up to 200 mM (see **FIG. 19**). The analysis of each fraction by SDS-PAGE confirmed the single chain Fv in fraction A and fraction B.

[0187] (3) Gel Filtration

[0188] Each of fractions A and B in (2) was separately concentrated with CentriPrep-10 and applied onto TSKgel G3000SWG column (21.5×600 mm) equilibrated with 20 mM acetate buffer (pH 6.0) containing 0.15 M NaCl. Chromatograms are shown in **FIG. 20**. The analysis of the fractions by SDS-PAGE confirmed that both major peaks (AI and BI) are of desired single chain Fv. In the gel filtration analysis, the fraction A was eluted at 36 kDa of apparent molecular weight and the fraction B was eluted at 76 kDa. The purified single chain Fvs (AI, BI) were analyzed with 15% SDS polyacrylamide gel. Samples were treated in the absence or presence of a reductant and the electrophoresis was carried out in accordance with the Laemmli's method. Then the protein was stained with Coomassie Brilliant Blue. As shown in **FIG. 21**, both AI and BI gave a single band at 35 kDa of apparent molecular-weight, regardless of the absence or presence of the reductant. From the above, it is concluded that AI is a monomer of the single chain Fv and BI is a non-covalently bound dimer of the single chain Fv. The gel filtration analysis of the fractions AI and BI with TSKgel G3000SW column (7.5×60 mm) revealed that a peak of the monomer is detected only in the fraction AI and a peak of the dimer is detected only in the fraction BI (**FIG. 22**). The dimer fraction (fraction BI) accounted for 4 period of total single chain Fvs.-More than 90% of the dimer in the dimer fraction was stably preserved for more than a month at 4° C.

[0189] 5.10 Construction of Vector Expressing Single Chain Fv Derived from Antibody MABL-2 in *E. coli* Cell

[0190] The pscM2 vector was modified by the PCR method to prepare a vector effectively expressing the single chain Fv from the antibody MABL-2 in *E. coli* cells. The resultant DNA fragment was introduced into pSCFVT7 expression vector.

[0191] As a forward primer for PCR, Nde-VHSm02 primer shown in SEQ ID No. 27 was designed to hybridize to a DNA encoding the N-terminal of the H chain V region and to contain a start codon and NdeI restriction enzyme recognition site. As a reverse primer for PCR, VLAS primer shown in SEQ ID No. 28 was designed to hybridize to a DNA encoding the C-terminal of the L chain V region and

to contain two stop codons and EcoRI restriction enzyme recognition site. The forward primer, Nde-VHSm02, comprises five point mutations in the part hybridizing to the DNA encoding the N-terminal of the H chain V region for the effective expression in *E. coli*.

[0192] 100 μ l of a PCR solution comprising 10 μ l of 10×PCR Buffer #1, 1 mM MgCl₂, 0.2 mM dNTPs, 5 units of KOD DNA polymerase (all from TOYOBO), 1 μ M of each primer and 100 ng of a template DNA (pscM2) was heated at 98° C. for 15 seconds, at 65° C. for 2 seconds and at 74° C. for 30 seconds in order. This temperature cycle was repeated 25 times.

[0193] The PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN) and digested by NdeI and EcoRI, and then the resulting DNA fragment was cloned into pSCFVT7 vector, from which pelB signal sequence had been eliminated by the digestion with NdeI and EcoRI. After DNA sequencing, the resulting plasmid comprising a DNA fragment with the desired DNA sequence is designated as "pscM2DEm02" (see **FIG. 23**). The nucleotide sequence and the amino acid sequence of the single chain Fv derived from the antibody MABL-2 contained in the plasmid pscM2DEm02 are shown in SEQ ID No. 29.

[0194] 5.11 Expression of Single Chain Fv Derived from Antibody MABL-2 in *E. coli* Cells

[0195] *E. coli* BL21(DE3)pLysS (STRATAGENE) was transformed with pscM2DEm02 vector to obtain a strain of *E. coli* expressing the single chain Fv derived from antibody MABL-2. The resulting clones were examined for the expression of the desired protein using SDS-PAGE, and a clone with a high expression level was selected as a strain producing the single chain Fv derived from antibody MABL-2.

[0196] 5.12 Purification of Single Chain Fv Derived from Antibody MABL-2 Produced in *E. coli*

[0197] A single colony of *E. coli* obtained by the transformation was cultured in 3 ml of LB medium at 28° C. for 7 hours and then in 70 ml of LB medium at 28° C. overnight. This pre-culture was transplanted to 7 L of LB medium and cultured at 28° C. with stirring at 300 rpm using the Jar-fermenter. When an absorbance of the medium reached O.D.=1.5, the bacteria were induced with 1 mM IPTG and then cultured for 3 hours.

[0198] The culture medium was centrifuged (10000×g, 10 minutes) and the precipitated bacteria were recovered. To the bacteria was added 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, 0.1 M NaCl and 1% Triton X-100 and the bacteria were disrupted by ultrasonication (out put: 4, duty cycle: 70%, 1 minutex10 times). The suspension of disrupted bacteria was centrifuged (12000×g, 10 minutes) to precipitate inclusion body. Isolated inclusion body was mixed with 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, 0.1 M NaCl and 4% Triton X-100, treated by ultrasonication (out put: 4, duty cycle: 50%, 30 secondsx2 times) again and centrifuged (12000×g, 10 minutes) to isolate the desired protein as precipitate and to remove containment proteins included in the supernatant.

[0199] The inclusion body comprising the desired protein was lysed in 50 mM Tris-HCl buffer (pH 8.0) containing 6 M Urea, 5 mM EDTA and 0.1 M NaCl and applied onto

Sephacryl S-300 gel filtration column (5×90 cm, Amersham Pharmacia) equilibrated with 50 mM Tris-HCl buffer (pH 8.0) containing 4M Urea, 5 mM EDTA, 0.1 M NaCl and 10 mM mercaptoethanol at a flow rate of 5 ml/minutes to remove associated single chain Fvs with high-molecular weight. The obtained fractions were analyzed with SDS-PAGE and the fractions with high purity of the protein were diluted with the buffer used in the gel filtration up to $O.D_{280}=0.25$. Then, the fractions were dialyzed three times against 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA, 0.1 M NaCl, 0.5 M Arg, 2 mM glutathione in the reduced form and 0.2 mM glutathione in the oxidized form in order for the protein to be refolded. Further, the fraction was dialyzed three times against 20 mM acetate buffer (pH 6.0) containing 0.15 M NaCl to exchange the buffer.

[0200] The dialysate product was applied onto Superdex 200 pg gel filtration column (2.6×60 cm, Amersham Pharmacia) equilibrated with 20 mM acetate buffer (pH 6.0) containing 0.15 M NaCl to remove a small amount of high molecular weight protein which was intermolecularly crosslinked by S—S bonds. As shown in FIG. 24, two peaks, major and sub peaks, were eluted after broad peaks which are expectedly attributed to an aggregate with a high molecular weight. The analysis by SDS-PAGE (see FIG. 21) and the elution positions of the two peaks in the gel filtration analysis suggest that the major peak is of the monomer of the single chain Fv and the sub peak is of the non-covalently bound dimer of the single chain Fv. The non-covalently bound dimer accounted for 4 percent of total single chain Fvs.

[0201] 5.13 Apoptosis-Inducing Activity In Vitro of Single Chain Fv Derived from Antibody MABL-2

[0202] An apoptosis-inducing action of the single chain Fv from antibody MABL-2 (MABL2-scFv) produced by the CHO cells and *E. coli* was examined according to two protocols by Annexin-V staining (Boehringer Mannheim) using the L1210 cells (hIAP/L1210) into which human IAP gene had been introduced.

[0203] In the first protocol sample antibodies at the final concentration of 3 $\mu\text{g}/\text{ml}$ were added to 5×10^4 cells of hIAP/L1210 cell line and cultured for 24 hours. Sample antibodies, i.e., the monomer and the dimer of the single chain Fv of MABL-2 from the CHO cells obtained in Example 5.9, the monomer and the dimer of the single chain Fv of MABL-2 from *E. coli* obtained in Example 5.12, and the mouse IgG antibody as a control were analyzed. After culturing, the Annexin-V staining was carried out and the fluorescence intensity thereof was measured using the FAC-Scan apparatus (BECTON DICKINSON).

[0204] In the second protocol sample antibodies at the final concentration of 3 $\mu\text{g}/\text{ml}$ were added to 5×10^4 cells of hIAP/L1210 cell line, cultured for 2 hours and mixed with anti-FLAG antibody (SIGMA) at the final concentration of 15 $\mu\text{g}/\text{ml}$ and further cultured for 22 hours. Sample antibodies of the monomer of the single chain Fv of MABL-2 from the CHO cells obtained in Example 5.9 and the mouse IgG antibody as a control were analyzed. After culturing, the Annexin-V staining was carried out and the fluorescence intensity thereof was measured using the FACScan apparatus.

[0205] Results of the analysis by the Annexin-V staining are shown in FIGS. 25-31. The results show that the dimers

of the single chain Fv polypeptide of MABL-2 produced in the CHO cells and *E. coli* remarkably induced cell death (FIGS. 26, 27) in comparison with the control (FIG. 25), while no apoptosis-inducing action was observed in the monomers of the single chain Fv polypeptide of MABL-2 produced in the CHO cells and *E. coli* (FIGS. 28, 29). When anti-FLAG antibody was used together, the monomer of the single chain Fv polypeptide derived from antibody MABL-2 produced in the CHO cells induced remarkably cell death (FIG. 31) in comparison with the control (FIG. 30).

[0206] 5.14 Antitumor Effect of the Monomer and the Dimer of scFv/CHO Polypeptide with a Model Mouse of Human Myeloma

[0207] (1) Quantitative Measurement of Human IgG in Mouse Serum

[0208] Measurement of human IgG (M protein) produced by human myeloma cell and contained in mouse serum was carried out by the following ELISA. 100 μL of goat anti-human IgG antibody (BIOSOURCE, Lot#7902) diluted to 1 $\mu\text{g}/\text{mL}$ with 0.1% bicarbonate buffer (pH 9.6) was added to each well on 96 wells plate (Nunc) and incubated at 4° C. overnight so that the antibody was immobilized. After blocking, 100 μL of the stepwisely diluted mouse serum or human IgG (CAPPEL, Lot#00915) as a standard was added to each well and incubated for 2 hours at a room temperature. After washing, 100 μL of alkaline phosphatase-labeled anti-human IgG antibody (BIOSOURCE, Lot#6202) which had been diluted to 5000 times was added, and incubation was carried out for 1 hour at a room temperature. After washing, a substrate solution was added. After incubation, absorbance at 405 nm was measured using the MICROPLATE READER Model 3550 (BioRad). The concentration of human IgG in the mouse serum was calculated based on the calibration curve obtained from the absorbance values of human IgG as the standard.

[0209] (2) Preparation of Antibodies for Administration

[0210] The monomer and the dimer of the scFv/CHO polypeptide were respectively diluted to 0.4 mg/mL or 0.25 mg/mL with sterile filtered PBS(-) on the day of administration to prepare samples for the administration.

[0211] (3) Preparation of a Mouse Model of Human Myeloma

[0212] A mouse model of human myeloma was prepared as follows. KPMM2 cells passaged in vivo (JP-Apl. 7-236475) by SCID mouse (Japan Clare) were suspended in RPMI1640 medium (GIBCO-BRL) containing 10% fetal bovine serum (GIBCO-BRL) and adjusted to 3×10^7 cells/mL. 200 μL of the KPMM2 cell suspension (6×10^6 cells/mouse) was transplanted to the SCID mouse (male, 6 week-old) via caudal vein thereof, which had been subcutaneously injected with the asialo GM1 antibody (WAKO JUNYAKU, 1 vial dissolved in 5 mL) a day before the transplantation.

[0213] (4) Administration of Antibodies

[0214] The samples of the antibodies prepared in (2), the monomer (250 μL) and the dimer (400 μL), were administered to the model mice of human myeloma prepared in (3) via caudal vein thereof. The administration was started from three days after the transplantation of KPMM2 cells and was carried out twice a day for three days. As a control, 200 μL

of sterile filtered PBS(-) was likewise administered twice a day for three days via caudal vein. Each group consisted of seven mice.

[0215] (5) Evaluation of Antitumor Effect of the Monomer and the Dimer of scFv/CHO Polypeptide with the Model Mouse of Human Myeloma

[0216] The antitumor effect of the monomer and the dimer of scFv/CHO polypeptide with the model mice of human myeloma was evaluated in terms of the change of human IgG (M protein) concentration in the mouse serum and survival time of the mice. The change of human IgG concentration was determined by measuring it in the mouse serum collected at 24 days after the transplantation of KPMM2 cells by ELISA described in the above (1). The amount of serum human IgG (M protein) in the serum of the PBS(-)-administered group (control) increased to about 8500 $\mu\text{g/mL}$, whereas the amount of human IgG of the scFv/CHO dimer-administered group was remarkably low, that is, as low as one-tenth or less than that of the control group. Thus, the results show that the dimer of scFv/CHO strongly inhibits the growth of the KPMM2 cells (FIG. 32). As shown in FIG. 33, a remarkable elongation of the survival time was observed in the scFv/CHO dimer-administered group in comparison with the PBS(-)-administered group.

2% in PBS(-) was prepared. Test samples were the antibody MABL-2, the monomer and the dimer of the single chain Fv polypeptide produced by the CHO cells, and the monomer and the dimer of the single chain Fv polypeptide produced by *E. coli*, and the control was mouse IgG (ZYMED). For the investigation of the hemagglutination effect, round bottom 96-well plates available from Falcon were used. 50 μL per well of the aforementioned antibody samples and 50 μL of the 2% erythrocyte suspension were added and mixed in the well. After incubation for 2 hours at 37° C., the reaction mixtures were stored at 4° C. overnight and the hemagglutination thereof was determined. As a control, 50 μL per well of PBS(-) was used and the hemagglutination test was carried out in the same manner. The mouse IgG and antibody MABL-2 were employed at 0.01, 0.1, 1.0, 10.0 or 100.0 $\mu\text{g/mL}$ of the final concentration of the antibodies. The single chain Fvs were employed at 0.004, 0.04, 0.4, 4.0, 40.0 or 80.0 $\mu\text{g/mL}$ of the final concentration and further at 160.0 $\mu\text{g/mL}$ only in the case of the dimer of the polypeptide produced by *E. coli*. Results are shown in the Table 2. In the case of antibody MABL-2, the hemagglutination was observed at a concentration of more than 0.1 $\mu\text{g/mL}$, whereas no hemagglutination was observed for both the monomer and the dimer of the single chain Fv.

TABLE 2

	Hemagglutination Test								
	Control	0.01	0.1	1	10	100	$\mu\text{g/mL}$		
mIgG	-	-	-	-	-	-			
MABL-2 (intact)	-	-	+	+++	+++	++			
	Control	0.004	0.04	0.4	4	40	80	$\mu\text{g/mL}$	
scFv/CHO monomer	-	-	-	-	-	-	-		
scFv/CHO dimer	-	-	-	-	-	-	-		
	Control	0.004	0.04	0.4	4	40	80	160	$\mu\text{g/mL}$
scFv/ <i>E. coli</i> monomer	-	-	-	-	-	-	-	-	
scFv/ <i>E. coli</i> dimer	-	-	-	-	-	-	-	-	

[0217] From the above, it is confirmed that the dimer of scFv/CHO has an antitumor effect for the human myeloma model mice. It is considered that the antitumor effect of the dimer of scFv/CHO, the modified antibody of the invention, results from the apoptosis-inducing action of the modified antibody.

[0218] 5.15 Hemagglutination Test

[0219] Hemagglutination test and determination of hemagglutination were carried out in accordance with "Immuno-Biochemical Investigation", zoku-Seikagaku Jikken Koza, edited by the Biochemical Society of Japan, published by Tokyo Kagaku Dojin.

[0220] Blood was taken from a healthy donor using heparin-treated syringes and washed with PBS(-) three times, and then erythrocyte suspension with a final concentration of

[0221] Example 6 Modified antibody sc(Fv)_2 comprising two H chain V regions and two L chain V regions and antibody MABL-2 scFvs having linkers with different length

[0222] 6.1 Construction of Plasmid Expressing Antibody MABL-2 sc(Fv)_2

[0223] For the preparation of a plasmid expressing the modified antibody [sc(Fv)_2] which comprises two H chain V 10 regions and two L chain V regions derived from the antibody MABL-2, the aforementioned pCHOM2, which comprises the DNA encoding scFv derived from the MABL-2 described above, was modified by the PCR method as mentioned below and the resulting DNA fragment was introduced into pCHOM2.

[0224] Primers employed for the PCR are EF1 primer (SEQ ID NO: 30) as a sense primer, which is designed to

hybridize to a DNA encoding EF1 α , and an antisense primer (SEQ ID NO: 19), which is designed to hybridize to the DNA encoding C-terminal of the L chain V region and to contain a DNA sequence coding for a linker region, and VLLAS primer containing SalI restriction enzyme recognition site (SEQ ID NO 31).

[0225] 100 μ l of the PCR solution comprises 10 μ l of 10 \times PCR Buffer #1, 1 mM MgCl₂, 0.2 mM dNTPs (dATP, dGTP, dCTP and dTTP), 5 units of KOD DNA polymerase (Toyobo, Inc.), 1 μ M of each primer and 100 ng of the template DNA (pCHOM2). The PCR solution was heated at 94° C. for 30 seconds, at 50° C. for 30 seconds and at 74° C. for 1 minute in order. This temperature cycle was repeated 30 times.

[0226] The PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN) and digested by SalI. The resultant DNA fragment was cloned into pBluescript KS⁺ vector (Toyobo, Inc.). After DNA sequencing, a plasmid comprising the desired DNA sequence was digested by SalI and the obtained DNA fragment was connected using Rapid DNA Ligation Kit (BOEHRINGER MANNHEIM) to pCHOM2 digested by SalI. After DNA sequencing, a plasmid comprising the desired DNA sequence is designated as "pCHOM2(Fv)₂" (see FIG. 34). The nucleotide sequence and the amino acid sequence of the antibody MABL-2 sc(Fv)₂ region contained in the plasmid pCHOM2(Fv)₂ are shown in SEQ ID No. 32.

[0227] 6.2 Preparation of Plasmid Expressing Antibody MABL-2 scFvs Having Linkers with Various Length

[0228] The scFvs containing linkers with different length and the V regions which are designed in the order of [H chain]-[L chain] (hereinafter "HL") or [L chain]-[H chain] (hereinafter "LH") were prepared using, as a template, cDNAs encoding the H chain and the L chain derived from the MABL-2 as mentioned below.

[0229] To construct HL type scFv the PCR procedure was carried out using pCHOM2(Fv)₂ as a template. In the PCR step, a pair of CFHL-F1 primer (SEQ ID NO: 33) and CFHL-R2 primer (SEQ ID NO: 34) or a pair of CFHL-F2 primer (SEQ ID NO: 35) and CFHL-R1 primer (SEQ ID NO: 36) and KOD polymerase were employed. The PCR procedure was carried out by repeating 30 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order to produce a cDNA for the H chain containing a leader sequence at 5'-end or a cDNA for the L chain containing FLAG sequence at 3'-end thereof. The resultant cDNAs for the H chain and the L chain were mixed and PCR was carried out by repeating 5 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order using the mixture as templates and the KOD polymerase. To the reaction mixture were added CFHL-F1 and CFHL-R1 primers and then the PCR reaction was performed by repeating 30 times of the aforementioned temperature cycle to produce a cDNA for HL-0 type without a linker.

[0230] To construct LH type scFv, the PCR reaction was carried out using, as a template, pGEM-M2L and pGEM-M2H which contain cDNAs encoding the L chain V region and the H chain V region from the antibody MABL-2, respectively (see JP-Appl. 11-63557). A pair of T7 primer (SEQ ID NO: 37) and CFLH-R2 primer (SEQ ID NO: 38) or

a pair of CFLH-F2 primer (SEQ ID NO: 39) and CFLH-R1 (SEQ ID NO: 40) and the KOD polymerase (Toyobo Inc.) were employed. The PCR reaction was performed by repeating 30 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in sequential order to produce a cDNA of an L chain containing a leader sequence at 5'-end or a cDNA of an H chain containing FLAG sequence at 3'-end thereof. The resultant cDNAs of the L chain and the H chain were mixed and PCR was carried out using this mixture as templates and the KOD polymerase by repeating 5 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order. To the reaction mixture were added T7 and CFLH-R1 primers and the reaction was performed by repeating 30 times of the aforementioned temperature cycle. The reaction product was used as a template and PCR was carried out using a pair of CFLH-F4 primer (SEQ ID NO: 41) and CFLH-R1 primer by repeating 30 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order to produce a cDNA of LH-0 type without a linker.

[0231] The resultant cDNAs of LH-0 and HL-0 types were digested by EcoRI and BamHI restriction enzymes (Takara Shuzo) and the digested cDNAs were introduced into an expression plasmid INPEP4 for mammalian cells using Ligation High (Toyobo Inc.), respectively. Competent *E. coli* JM109 (Nippon Gene) was transformed with each plasmid and the desired plasmids were isolated from the transformed *E. coli* using QIAGEN Plasmid Maxi Kit (QIAGEN). Thus plasmids pCF2LH-0 and pCF2HL-0 were prepared.

[0232] To construct the expression plasmids of HL type containing linkers with different size, pCF2HL-0, as a template, and CFHL-X3 (SEQ ID NO: 42), CFHL-X4 (SEQ ID NO: 43), CFHL-X5 (SEQ ID NO: 44), CFHL-X6 (SEQ ID NO: 45) or CFHL-X7 (SEQ ID NO: 46), as a sense primer, and BGH-1 (SEQ ID NO: 47) primer, as an antisense primer, which is complementary with the vector sequence were employed. PCR reaction was carried out using the KOD polymerase by repeating 30 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order and the reaction products were digested by restriction enzymes XhoI and BamHI (Takara Shuzo). The digested fragments were introduced between XhoI and BamHI sites in the pCF2HL-0 using Ligation High (Toyobo Inc.), respectively. Competent *E. coli* JM109 was transformed with each plasmid and the desired plasmids were isolated from the transformed *E. coli* by using Qiagen Plasmid Maxi kit. Thus expression plasmids pCF2HL-3, pCF2HL-4, pCF2HL-5, pCF2HL-6 and pCF2HL-7 were prepared.

[0233] To construct expression plasmid for the transient expression in COS7 cells the plasmids pCF2HL-0, pCF2HL-3, pCF2HL-4, pCF2HL-5, pCF2HL-6 and pCF2HL-7 were digested by restriction enzymes EcoRI and BamHI (Takara Shuzo) and the resultant fragments of approximately 800 bp were purified with agarose gel electrophoresis. The obtained fragments were introduced between EcoRI and BamHI sites in an expression plasmid pCOS1 for the expression in mammalian cells by using Ligation High (Toyobo Inc.), respectively. Competent *E. coli* DH5 α (Toyobo Inc.) was transformed with each plas-

mid and the desired plasmids were isolated from the transformed *E. coli* using Qiagen Plasmid Maxi kit. Thus the expression plasmids CF2HL-0/pCOS1, CF2HL-3/pCOS1, CF2HL-4/pCOS1, CF2HL-5/pCOS1, CF2HL-6/pCOS1 and CF2HL-7/pCOS1 were prepared.

[0234] As a typical example of these plasmids, the construction of the plasmid CF2HL-0/pCOS1 is illustrated in FIG. 35 and the nucleotide sequence and the amino acid sequence of MABL2-scFv.<HL-0> contained in the plasmid are shown in SEQ ID No. 48. Nucleotide sequences and amino acid sequences of the linker regions in these plasmids are also shown in FIG. 36.

[0235] To construct the expression plasmids of LH type containing linkers with different size, pCF2LH-0, as a template, and CFLH-X3 (SEQ ID NO: 49), CFLH-X4 (SEQ ID NO: 50), CFLH-X5 (SEQ ID NO: 51), CFLH-X6 (SEQ ID NO: 52) or CFLH-X7 (SEQ ID NO: 53), as a sense primer, and BGH-1 primer, as an antisense primer, which is complementary with the vector sequence were employed. PCR reaction was carried out using the KOD polymerase by repeating 30 times the temperature cycle consisting of 94° C. for 30 seconds, 60° C. for 30 seconds and 72° C. for 1 minute in order and the reaction products were digested by restriction enzymes XhoI and BamHI. The digested fragments were introduced into the pCF2LH-0 between XhoI and BamHI sites using Ligation High, respectively. Competent *E. coli* DH5 α (Toyobo Inc.) was transformed with each plasmid and the desired plasmids were isolated from the transformed *E. coli* using Qiagen Plasmid Maxi kit. Thus expression plasmids pCF2LH-3, pCF2LH-4, pCF2LH-5, pCF2LH-6 and pCF2LH-7 were prepared.

[0236] To construct expression plasmid for the transient expression in COS7 cells the plasmids pCF2LH-0, pCF2LH-3, pCF2LH-4, pCF2LH-5, pCF2LH-6 and pCF2LH-7 were digested by restriction enzymes EcoRI and BamHI (Takara Shuzo) and the resultant fragments of approximately 800 bp were purified with agarose gel electrophoresis. The obtained fragments were introduced between XhoI and BamHI sites in an expression plasmid pCOS1 for the expression in mammalian cells by using the Ligation High, respectively. Competent *E. coli* DH5 α (Toyobo Inc.) was transformed with each plasmid and the desired plasmids were isolated from the transformed *E. coli* using the Qiagen Plasmid Maxi kit. Consequently, the expression plasmids CF2LH-0/pCOS1, CF2LH-3/pCOS1, CF2LH-4/pCOS1, CF2LH-5/pCOS1, CF2LH-6/pCOS1 and CF2LH-7/pCOS1 were prepared.

[0237] As a typical example of these plasmids, the construction of the plasmid CF2LH-0/pCOS1 is illustrated in FIG. 37 and the nucleotide sequence and the amino acid sequence of MABL2-scFv <LH-0> contained in the plasmid are shown in SEQ ID No. 54. Nucleotide sequences and amino acid sequences of the linker regions in these plasmids are also shown in FIG. 38.

[0238] 6.3 Expression of scFvs and sc(Fv)₂ in COS7 Cells

[0239] (1) Preparation of Culture Supernatant Using Serum-Containing Culture Medium

[0240] The HL type and LH type of scFvs and sc(Fv)₂ were transiently expressed in COS7 cells (JCRB9127, Japan Health Sciences-Foundation). COS7 cells were subcultured in DMEM media (GIBCO BRL) containing 10% fetal

bovine serum (HyClone) at 37° C. in carbon dioxide atmosphere incubator. The COS7 cells were transfected with CF2HL-0, 3~7/pCOS1, or CF2LH-0, 3~7/pCOS1 prepared in Example 6.2 or pCHOM2(Fv)₂ vectors by electroporation using the Gene Pulser apparatus (BioRad). The DNA (10 μ g) and 0.25 ml of 2 \times 10⁷ cells/ml in DMEM culture medium containing 10% FBS and 5 mM BES (SIGMA) were added to a cuvette. After standing for 10 minutes the mixtures were treated with pulse at 0.17 kV, 950 μ F of electric capacity. After the restoration for 10 minutes at room temperature, the electroporated cells were transferred into the DMEM culture medium (10%FBS) in 75 cm³ flask. After culturing for 72 hours, the culture supernatant was collected and centrifuged to remove cell fragments. The culture supernatant was subjected to the filtration using 0.22 μ m bottle top filter (FALCON) to obtain the culture supernatant (hereinafter "CM").

[0241] (2) Preparation of Culture Supernatant Using Serum-Free Culture Medium

[0242] Cells transfected in the same manner as (1) were transferred to the DMEM medium (10% FBS) in 75 cm³ flask and cultured overnight. After the culture, the supernatant was discarded and the cells were washed with PBS and then added to CHO-S-SFM II medium (GIBCO BRL). After culturing for 72 hours, the culture supernatant was collected, centrifuged to remove cell fragments and filtered using 0.22 μ m bottle top filter (FALCON) to obtain CM.

[0243] 6.4 Detection of scFvs and sc(Fv)₂ in CM of COS7

[0244] The various MABL2-scFVs and sc(Fv)₂ in CM of COS7 prepared in the aforementioned Example 6.3 (2) were detected by Western Blotting method.

[0245] Each CM of COS7 was subjected to SDS-PAGE electrophoresis and transferred to REINFORCED NC membrane (Schleicher & Schuell). The membrane was blocked with 5% skim milk (Morinaga Nyu-gyo) and washed with TBS. Then an anti-FLAG antibody (SIGMA) was added thereto. The membrane was incubated at room temperature and washed. A peroxidase labeled mouse IgG antibody (Jackson Immuno Research) was added. After incubating and washing at room temperature, the substrate solution (Kirkegaard Perry Laboratories) was added to develop color (FIG. 39).

[0246] 6.5 Flow Cytometry

[0247] Flow cytometry was performed using the culture supernatants of COS7 cells prepared in Example 6.3 (1) to measure the binding of the MABL2-scFVs and sc(Fv)₂ to human Integrin Associated Protein (IAP) antigen. The culture supernatants to be tested or a culture supernatant of COS7 cells as a control was added to 2 \times 10⁵ cells of the mouse leukemia cell line L1210 expressing human IAP. After incubating on ice and washing, 10 μ g/mL of the mouse anti-FLAG antibody (SIGMA) was added and then the cells were incubated and washed. Then, the FITC labeled anti-mouse IgG antibody (BECTON DICKINSON) was added thereto and the cells were incubated and washed again. The fluorescence intensity was measured using the FACScan apparatus (BECTON DICKINSON). The results of the flow cytometry show that the MABL2-scFvs having linkers with different length and the sc(Fv)₂ in the culture supernatants of COS7 have high affinity to human IAP (see FIGS. 40a and 40b).

[0248] 6.6 Apoptosis-Inducing Effect In Vitro

[0249] An apoptosis-inducing action of the culture supernatants of COS7 prepared in Example 6.3 (1) was examined by Annexin-V staining (Boehringer Mannheim) using the L1210 cells transfected with human IAP gene (hIAP/L1210).

[0250] To 5×10^4 cells of the hIAP/L1210 cells were added the culture supernatants of COS7 cells transfected with each vectors or a culture supernatant of COS7 cells as a control at 10% of the final concentration and the mixtures were cultured for 24 hours. Then, the Annexin-V/PI staining was performed and the fluorescence intensity was measured using the FACScan apparatus (BECTON DICKINSON). The results revealed that scFvs <HL3, 4, 6, 7, LH3, 4, 6, 7> and sc(Fv)₂ in CM of COS7 induced remarkable cell death of hIAP/L1210 cells. These results are shown in **FIG. 41**.

[0251] 6.7 Construction of Vectors for the Expression of scFvs and sc(Fv)₂ in CHO Cells

[0252] To isolate and purify MABL2-scFvs and sc(Fv)₂ from culture supernatant, the expression vectors for expressing in CHO cells were constructed as below.

[0253] The EcoRI-BamHI fragments of pCF2HL-0, 3~7, and pCF2LH-0, 3~7 prepared in Example. 6.2 were introduced between EcoRI and BamHI sites in an expression vector pCHO1 for CHO cells using the Ligation High. Competent *E. coli* DH5 α was transformed with them. The plasmids were isolated from the transformed *E. coli* using QIAGEN Plasmid Midi kit-(QIAGEN) to prepare expression plasmids pCHOM2HL-0, 3~7, and pCHOM2LH-0, 3~7.

[0254] 6.8 Production of CHO Cells Expressing MABL2-scFvs <HL-0, 3~7>, MABL2-scFvs <LH-0, 3~7> and sc(Fv)₂ and Preparation of the Culture Supernatants Thereof

[0255] CHO cells were transformed with each of the expression plasmids pCHOM2HL-0, 3~7, and pCHOM2LH-0, 3~7, constructed in Example 6.7 and pCHOM2(Fv)₂ vector to prepare the CHO cells constantly expressing each modified antibody. As a typical example thereof, the production of the CHO cells constantly expressing MABL2-scFv <HL-5> or sc(Fv)₂ is illustrated as follows.

[0256] The expression plasmids pCHOM2HL-5 and pCHOM2(Fv)₂ were linearized by digesting with a restriction enzyme PvuI and subjected to transfection to CHO cells by electroporation using Gene Pulser apparatus (BioRad). The DNA (10 μ g) and 0.75 ml of PBS with 1×10^7 cells/ml were added to a cuvette and treated with pulse at 1.5 kV, 25 μ F of electric capacity. After the restoration for 10 minutes at room temperature, the electroporated cells were transferred into nucleic acid-containing α -MEM culture medium (GIBCO BRL) containing 10% fetal bovine serum and cultured. After culturing overnight, the supernatant was discarded. The cells were washed with PBS and added to nucleic acid-free α -MEM culture medium (GIBCO BRL) containing 10% fetal bovine serum. After culturing for two weeks, the cells were cultured in a medium containing 10 nM (final concentration) methotrexate (SIGMA), then 50 nM and 100 nM methotrexate. The resultant cells were cultured in serum-free CHO-S-SFM II medium (GIBCO BRL) in a roller bottle. The culture supernatant was col-

lected, centrifuged to remove cell fragments and filtered using a filter with 0.22 μ m of pore size to obtain CM, respectively.

[0257] According to the above, CHO cells which constantly express MABL2-scFvs <HL-0, -3, -4, -6, -7> and <LH-0, -3, -4, -5, -6, -7> and CMs thereof were obtained.

[0258] 6.9 Purification of Dimer of MABL2-scFv <HL-5> and sc(Fv)₂

[0259] The MABL2-scFv <HL-5> and the sc(Fv)₂ were purified from CMs prepared in Example 6.8 by two types of purification method as below.

[0260] <Purification Method 1>

[0261] HL-5 and sc(Fv)₂ were purified by the anti-FLAG antibody affinity column chromatography utilizing the FLAG sequence located at C-terminal of the polypeptides and by gel filtration. One liter of CM as obtained in 6.8 was applied onto a column (7.9 ml) prepared with anti-FLAG M2 Affinity gel (SIGMA) equilibrated with 50 mM Tris-HCl buffer (TBS, pH 7.5) containing 150 mM NaCl. After washing the column with TBS, the scFv was eluted by 0.1 M glycine-HCl buffer, pH 3.5. The resultant fractions were analyzed by SDS-PAGE and the elution of the scFv was confirmed. The scFv fraction was mixed with Tween 20 up to 0.01% of the final concentration and concentrated using Centricon-10 (MILIPORE). The concentrate was applied onto TSKgel G3000SWG column (7.5 \times 600 mm) equilibrated with 20 mM acetate buffer (pH 6.0) containing 150 mM NaCl and 0.01% Tween 20. At 0.4 mL/minute of the flow rate, the scFv was detected by the absorption at 280 nm. The HL-5 was eluted as the major fraction in the position of the dimer and the sc(FV)₂ was eluted in the position of the monomer.

[0262] <Purification Method 2>

[0263] HL-5 and sc(Fv)₂ were purified using three steps comprising ion exchange chromatography, hydroxyapatite and gel filtration. In the ion exchange chromatography, Q sepharose fast flow column (Pharmacia) was employed for HL-5 and SP-sepharose fast flow column was employed for sc(Fv)₂. In and after the second step, HL-5 and sc(FV)₂ were processed by the same procedure.

[0264] First Step for HL-5

[0265] CM of HL-5 was diluted to two times with 20 mM Tris-HCl buffer (pH 9.0) containing 0.02% Tween 20 and then the pH was adjusted to 9.0 with 1 M Tris. The solution was applied onto Q Sepharose fast flow column equilibrated with 20 mM Tris-HCl buffer (pH 8.5) containing 0.02% Tween 20. A polypeptide adsorbed to the column was eluted by a linear gradient of NaCl in the same-buffer, from 0.1 to 0.55 M. Monitoring the eluted fractions by SDS-PAGE, the fractions containing HL-5 were collected and subjected to hydroxyapatite of the second step.

[0266] First Step for sc(Fv)₂

[0267] CM of the sc(Fv)₂ was diluted to two times with 20 mM acetate buffer (pH 5.5) containing 0.02% Tween 20 and its pH was adjusted to 5.5 with 1 M acetic acid. The solution was applied onto a SP-Sepharose fast flow column equilibrated with 20 mM acetate buffer (pH 5.5) containing 0.02% Tween 20. A polypeptide adsorbed to the column was eluted by a linear gradient of NaCl in the buffer, from 0 to 0.5 M.

TABLE 3-continued

		Diluent: PBS ($\mu\text{g/ml}$)								
		cont	80	40	20	10	5	2.5	1.25	0.625
MABL2 (intact)	-	+	+	+	+	+	+	+	+	+
mlgG	-	-	-	-	-	-	-	-	-	-
		Diluent: Acetate Buffer ($\mu\text{g/ml}$)								
		cont	80	40	20	10	5	2.5	1.25	0.625
MABL2 (intact)	-	+	+	+	+	+	+	+	+	+
		Diluent: PBS ($\mu\text{g/ml}$)								
		0.1129	0.0564	0.0282	0.0141	0.0071	0.0035	0.0018		
MABL2-sc(Fv) ₂	-	-	-	-	-	-	-	-	-	-
		0.1094	0.0547	0.0273	0.0137	0.0068	0.0034	0.0017		
MABL2-sc(Fv) <HL5>	-	-	-	-	-	-	-	-	-	-
		0.3125	0.1563	0.0781	0.0391	0.0195	0.0098	0.0049		
MABL2 (intact) mlgG	+	±	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-
		Diluent: Acetate Buffer ($\mu\text{g/ml}$)								
		0.3125	0.1563	0.0781	0.0391	0.0195	0.0098	0.0049		
MABL2 (intact)	+	+	+	-	-	-	-	-	-	-

[0283] 6.13 Antitumor Effect of the Purified Dimer of scFv <HL-5> and the SC(FV)₂ for a Model Mouse of Human Myeloma

[0284] The antitumor effects were tested for the dimer of scFv <HL-5> and the sc(Fv)₂ prepared and purified in Examples 6.8 and 6.9. The test was performed by using the mouse model for human myeloma produced in Example 5.1 and determining the amount of M protein produced by human myeloma cells in the mouse serum using ELISA and examining survival time of the mice. Then, the antitumor effects of the dimer of scFv <HL-5> and the sc(Fv)₂ were evaluated in terms of the change of the amount of M protein in the mouse serum and the survival time of the mice.

[0285] In the test, the HL-5 and the sc(Fv)₂ were employed as a solution at 0.01, 0.1 or 1 mg/mL in vehicle consisting of 150 mM NaCl, 0.02% Tween and 20 mM acetate buffer, pH 6.0 and administered to the mice at 0.1, 1 or 10 mg/kg of dosage. Control group of mice were administered only with the vehicle.

[0286] The mouse serum was gathered 26 days after the transplantation of the human myeloma cells and the amount of M protein in the serum was measured using ELISA according to Example 5.14. As a result, the amount of M protein in the serum of both mice groups administered with HL-5, the dimer and the sc(Fv)₂ decreased in dose-dependent manner (see FIG. 44). Furthermore, a significant elongation of the survival time was observed in both groups

administered with the HL-5 (FIG. 45) and with the sc(Fv)₂ (FIG. 46) in comparison with the control group administered with the vehicle. These results show that the HL-5 and the sc(Fv)₂ of the invention have excellent antitumor effect in vivo.

EXAMPLE 7

Single Chain Fv Comprising H Chain V Region and L Chain V Region of Human Antibody 12B5 Against Human MPL

[0287] A DNA encoding V regions of human monoclonal antibody 12B5 against human MPL was constructed as follows:

[0288] 7.1 Construction of a Gene Encoding H Chain V Region of 12B5

[0289] The gene encoding H chain V region of human antibody 12B5 binding to human MPL was designed by connecting the nucleotide sequence of the gene thereof (SEQ ID NO: 55) at the 5'-end to the leader sequence (SEQ ID NO: 56) originated from human antibody gene (Eur. J. Immunol. 1996; 26: 63-69). The designed nucleotide sequence was divided into four oligonucleotides having overlapping sequences of 15 bp each (12B5VH-1, 12B5VH-2, 12B5VH-3, 12B5VH-4). 12B5VH-1 (SEQ ID NO: 57) and 12B5VH-3 (SEQ ID NO: 59) were synthesized in the sense direction, and 12B5VH-2 (SEQ ID NO: 58) and

12B5VH-4 (SEQ ID NO: 60) in the antisense direction, respectively. After assembling each synthesized oligonucleotide by respective complementarity, the outside primers (12B5VH-S and 12B5VH-A) were added to amplify the full length of the gene. 12B5VH-S (SEQ ID NO: 61) was designed to hybridize to 5'-end of the leader sequence by the forward primer and to have Hind III restriction enzyme recognition site and Kozak sequence, and 12B5VH-A (SEQ ID NO: 62) was designed to hybridize to the nucleotide sequence encoding C-terminal of H chain V region by the reverse primer and to have a splice donor sequence and BamHI restriction enzyme recognition site, respectively.

[0290] 100 μ l of the PCR solution containing 5 μ l of 10 \times PCR Gold Buffer II, 1.5 mM MgCl₂, 0.08 mM dNTPs (dATP, dGTP, dCTP, dTTP), 5 units of DNA-polymerase AmpliTaq Gold (all by PERKIN ELMER) and each 2.5 μ l of each synthesized oligonucleotide (12B5VH-1 to -4) was heated at 94° C. of the initial temperature for 9 minutes, at 94° C. for 2 minutes, at 55° C. for 2 minutes and 72° C. for 2 minutes. After repeating the cycle two times each 100 pmole of external primer 12B5VH-S and 12B5VH-A was added. The mixture was subjected to the cycle consisting of at 94° C. for 30 seconds, at 55° C. for 30 seconds and 72° C. for 1 minute 35 times and heated at 72° C. for further 5 minutes.

[0291] The PCR product was purified by 1.5% low-melting-temperature agarose gel (Sigma), digested by restriction enzymes BamHI and Hind III, and cloned into expression vector HEF-gy1 for human H chain. After determining the DNA sequence the plasmid containing the correct DNA sequence was named HEF-12B5H-gy1.

[0292] The HEF-12B5H-gy1 was digested by restriction enzymes EcoRI and BamHI to produce the gene encoding 12B5VH which was then cloned into an expression vector pCOS-Fd for human Fab H chain to produce pFd-12B5H. The expression vector for human Fab H chain was constructed by amplifying the DNA (SEQ ID NO: 63) containing the intron region existing between the genes encoding human antibody H chain V region and the constant region, and the gene encoding a part of the constant region of human H chain by PCR, and inserting the PCR product into animal cell expression vector pCOS1. The human H chain constant region was amplified for the gene under the same conditions mentioned above using as the template HEF-gy1, as the forward primer G1CH1-S (SEQ ID NO: 64) which was designed to hybridize to 5'-end sequence of intron 1 and to have restriction enzyme recognition sites EcoRI and BamHI and as the reverse primer G1CH1-A (SEQ ID NO: 65) which was designed to hybridize to 3'-end DNA of human H chain constant region CH1 domain and to have a sequence encoding a part of hinge region, two stop codons and restriction enzyme recognition site Bgl II.

[0293] The nucleotide sequence and amino acid sequence of the reconstructed 12B5H chain variable region which were included in plasmids HEF-12B5H-gy1 and pFd-12B5H are shown in SEQ ID NO: 66.

[0294] 7.2 Construction of the Gene Encoding 12B5 L Chain V Region

[0295] The gene encoding L chain V region of human antibody 12B5 binding to human MPL was designed by connecting the nucleotide sequence of gene (SEQ ID-NO:

67) at the 5'-end to the leader sequence (SEQ ID NO: 68) originated from human antibody gene 3D6 (Nuc. Acid Res. 1990: 18; 4927). In the same way as mentioned above the designed nucleotide sequence was divided into four oligonucleotides having overlapping sequences of 15 bp each (12B5VL-1, 12B5VL-2, 12B5VL-3, 12B5VL-4) and synthesized respectively. 12B5VL-1 (SEQ ID NO: 69) and 12B5VL-3 (SEQ ID NO: 71) had sense sequences, and 12B5VL-2 (SEQ ID NO: 70) and 12B5VL-4 (SEQ ID NO: 72) had antisense sequences, respectively. Each of the synthesized oligonucleotides was assembled by respective complementarity and mixed with the external primer (12B5VL-S and 12B5VL-A) to amplify the full length of the gene. 12B5VL-S (SEQ ID NO: 73) was designed to hybridize to 5'-end of the leader sequence by the forward primer and to have Hind III restriction enzyme recognition site and Kozak sequence. 12B5VL-A (SEQ ID NO: 74) was designed to hybridize to the nucleotide sequence encoding C-terminal of L chain V region by the reverse primer and to have a splice donor sequence and BamHI restriction enzyme recognition site.

[0296] Performing the PCR as mentioned above, the PCR product was purified by 1.5% low-melting-temperature agarose gel (Sigma), digested by restriction enzymes BamHI and Hind III, and cloned into an expression vector HEF-gk for human L chain. After determining the DNA sequence the plasmid containing the correct DNA sequence was named HEF-12B5L-gk. The nucleotide sequence and amino acid sequence of the reconstructed 12B5 L chain V region which were included in plasmid HEF-12B5L-gk are shown in SEQ ID NO:75.

[0297] 7.3 Production of Reconstructed 12B5 Single Chain Fv (scFv)

[0298] The reconstructed 12B5 antibody single chain Fv was designed to be in the order of 12B5VH-linker-12B5VL and to have FLAG sequence (SEQ ID NO: 76) at C-terminal to facilitate the detection and purification. The reconstructed 12B5 single chain Fv (sc12B5) was constructed using a linker sequence consisting of 15 amino acids represented by (Gly₄Ser)₃.

[0299] (1) Production of the Reconstructed 12B5 Single Chain Fv Using the Linker Sequence Consisting of 15 Amino Acids

[0300] The gene encoding the reconstructed 12B5 antibody single chain Fv, which contained the linker sequence consisting of 15 amino-acids, was constructed by connecting 12B5 H chain V region, linker region and 12B5 L chain V region which was amplified by PCR respectively. This method is schematically shown in FIG. 47. Six PCR primers (A-F) were used for production of the reconstructed 12B5 single chain Fv. Primers A, C, and E had sense sequences, and primers B, D, and F had antisense sequences.

[0301] The forward primer 12B5-S (Primer A, SEQ ID NO: 77) for H chain V region was designed to hybridize to 5'-end of H chain leader sequence and to have EcoRI restriction enzyme recognition site. The reverse primer HuVHJ3 (Primer B, SEQ ID NO: 78) for H chain V region was designed to hybridize to DNA encoding C-terminal of H chain V region.

[0302] The forward primer RHuJH3 (Primer C, SEQ ID NO: 79) for the linker was designed to hybridize to DNA

encoding the N-terminal of the linker and to overlap DNA encoding the C-terminal of H chain V region. The reverse primer RHuVK1 (Primer D, SEQ ID NO: 80) for the linker was designed to hybridize to DNA encoding the C-terminal of the linker and overlap DNA encoding the N-terminal of L chain V region.

[0303] The forward primer HuVK1.2 (Primer E, SEQ ID NO: 81) for L chain V region was designed to hybridize to DNA encoding the N-terminal of L chain V region. The reverse primer 12B5F-A for L chain V region (Primer F, SEQ ID NO: 82) was designed to hybridize to DNA encoding C-terminal of L chain V region and to have the sequence encoding FLAG peptide (Hopp, T. P. et al., *Bio/Technology*, 6, 1204-1210, 1988), two transcription stop codons and NotI restriction enzyme recognition site.

[0304] In the first PCR step, three reactions A-B, C-D, and E-F were performed, and the three PCR products obtained from the first step PCR were assembled by respective complementarity. After adding primers A and F the full length DNA encoding the reconstructed 12B5 single chain Fv having the linker consisting of 15 amino acids was amplified (the second PCR). In the first step PCR, the plasmid HEF-12B5H-gy1 (see Example 7. 1) encoding the reconstructed 12B5 H chain V region, pSCFVT7-hM21 (humanized ONS-M21 antibody) (Ohtomo et al., *Anticancer Res.* 18 (1998), 4311-4316) containing DNA (SEQ ID NO: 83) encoding the linker region consisting of Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (Huston et al., *Proc. Natl. Acad. Sci. USA*, 85, 5879-5883, 1988) and the plasmid HEF-12B5L-gk (see Example 7. 2) encoding the reconstructed 12B5 L chain V region were used as templates, respectively.

[0305] 50 μ l of PCR solution for the first step contained 5 μ l of 10 \times PCR Gold Buffer II, 1.5 mM MgCl₂, 0.08 mM dNTPS, 5 units of DNA polymerase AmpliTaq Gold (all by PERKIN ELMER), each 100 pmole of each primer and 100 ng of each template DNA. The PCR solution was heated at 94° C. of the initial temperature for 9 minutes, at 94° C. for 30 seconds, 55° C. for 30 seconds and 72° C. for 1 minute. After repeating the cycle 35 times the reaction mixture was further heated 72° C. for 5 minutes.

[0306] The PCR products A-B, C-D, and E-F were assembled by the second PCR. PCR mixture solution for the second step of 98 μ l containing as the template 1 μ l of the first PCR product A-B, 0.5 μ l of PCR product C-D and 1 μ l of PCR product E-F, 10 μ l of 10 \times PCR Gold Buffer II, 1.5 mM MgCl₂, 0.08 mM dNTPs, 5 units of DNA polymerase AmpliTaq Gold (all by PERKIN ELMER) was heated at 94° C. of the initial temperature for 9 minutes, at 94° C. for 2 minutes, at 65° C. for 2 minutes and 72° C. for 2 minutes. After repeating the cycle two times, each 100 pmole of each of primers A and F were added. After repeating the cycle consisting of at 94° C. for 30 seconds, 55° C. for 30 seconds and 72° C. for 1 minute 35 times, the reaction mixture was heated at 72° C. for 5 minutes.

[0307] The DNA fragments produced by the second PCR were purified using 1.5% low-melting-temperature agarose gel, digested by EcoRI and NotI, and cloned into pCHO1 vector and pCOS1 vector (Japanese Patent Application No. 8-255196). The expression vector pCHO1 was a vector constructed by deleting the antibody gene from DHFR-AE-rvH-PM1-f (see WO92/19759) by EcoRI and SmaI diges-

tion, and connecting to EcoRI-NotI-BamHI Adaptor (TAKARA SHUZO). After determining the DNA sequence the plasmids containing the DNA fragment encoding the correct amino acid sequence of reconstructed 12B5 single chain Fv were named pCHO-sc12B5 and pCOS-sc12B5. The nucleotide sequence and amino acid sequence of the reconstructed 12B5 single chain Fv included in the plasmids pCHO-sc12B5 and pCOS-sc12B5 are shown in SEQ ID NO: 84.

[0308] 7.4 Expression of Antibody 12B5 (IgG, Fab) and Single Chain Fv Polypeptide by Animal Cell

[0309] Antibody 12B5 (IgG, Fab) and single chain Fv derived from antibody 12B5 were expressed by using COS-7 cells or CHO cells.

[0310] The transient expression using COS-7 cells was performed as follows. The transfection was performed by electroporation method using Gene Pulser equipment (BioRad). For the expression of antibody 12B5 (IgG) each 10 μ g of the above-mentioned expression vector HEF-12B5H-gy1 and HEF-12 B5L-gk were added, for the expression of 12B5Fab fragment each 10 μ g of pFd-12B5H and HEF-12B5L-gk were added and for the expression of single chain Fv 10 μ g of pCOS-sc12B5 was added to COS-7 cells (1 \times 10⁷ cells/ml) suspended in 0.8 ml of PBS. The mixture kept in a cuvette was treated by pulse at the capacity of 1.5 kV, 25 μ FD. After recovering for 10 minutes in a room temperature the electroporated cells were added to DMEM culture medium (GIBCO BRL) containing 10% bovine fetal serum cultivated. After cultivating overnight the cells were washed once by PBS, added to serum-free medium CHO-S-SFM II and cultivated for 2 days. The culture medium was centrifuged to remove cell debris and filtered with 0.22 μ m filter to prepare the culture supernatant.

[0311] To establish a stable expression CHO cell line for the single chain Fv (polypeptide) derived from antibody 12B5, the expression vector pCHO-sc12B5 was introduced into CHO cells as follows.

[0312] The expression vector was introduced into CHO cells by electroporation method using Gene Pulser equipment (BioRad). Linearized DNA (100 μ g) obtained by digestion with restriction enzyme PvuI and CHO cells (1 \times 10⁶ cells/ml) suspended in 0.8 ml of PBS were mixed in a cuvette, left stationary on ice for 10 minutes and treated with pulse at the capacity of 1.5 kV, 25 μ FD. After recovering for 10 minutes at a room temperature the electroporated cells were added to CHO-S-SFM II (GIBCO BRL) containing 10% bovine fetal serum and cultivated. After cultivating for 2 days the cultivation was continued in CHO-S-SFM II (GIBCO BRL) containing 5 nM methotrexate (SIGMA) and 10% bovine fetal serum. From thus obtained clones a clone with high expression rate was selected as the production cell line for 12B5 single chain Fv. After cultivating in serum-free medium CHO-S-SFM II (GIBCO BRL) containing 10 nM methotrexate (SIGMA), the culture supernatant was obtained by centrifugal separation of cell debris.

[0313] 7.5 Purification of Single Chain Fv Derived from 12B5 Produced by CHO Cells

[0314] The culture supernatant of CHO cell line expressing 12B5 single chain Fv obtained in 7.4 was purified by anti-FLAG antibody column and gel filtration column.

[0315] (1) Anti-FLAG Antibody Column

[0316] The culture supernatant was added to anti-FLAG M2 affinity gel (SIGMA) equilibrated by PBS. After washing the column by the same buffer the proteins adsorbed to the column were eluted by 0.1M glycine-HCl buffer (pH 3.5). The eluted fractions were immediately neutralized by adding 1 M Tris-HCl buffer (pH 8.0). The eluted fractions were analyzed by SDS-PAGE and the fraction which was confirmed to contain the single chain Fv was concentrated using Centricon-10 (MILLIPORE).

[0317] (2) Gel Filtration

[0318] The concentrated solution obtained in (1) was added to Superdex200 column (10×300 mm, AMERSHAM PHARMACIA) equilibrated by PBS containing 0.01% Tween20. The product sc12B5 was eluted in two peaks (A, B) (see FIG. 48). The fractions A and B were analyzed using the 14%-SDS-polyacrylamide gel. The sample was processed by electrophoresis in the presence and absence of a reducing agent according to Laemmli method, and stained by Coomassie Brilliant Blue after the electrophoresis. As shown in FIG. 49 the fractions A and B, regardless of the presence of the reducing agent or its absence, produced a single band having an apparent molecular weight of about 31 kD. When the fractions A and B were analyzed by gel filtration using Superdex200 PC 3.2/30 (3.2×300 mm, AMERSHAM PHARMACIA), the fraction A produced an eluted product at an apparent molecular weight of about 44 kD and the fraction B produced at 22kD (see FIGS. 50a and b). The results show that the fraction A is the non-covalent bond dimer of sc12B5 single chain Fv, and B is the monomer.

[0319] 7.6 Measurement of TPO-Like Agonist Activity of Various Single Chain Fvs

[0320] The TPO-like activity of anti-MPL single chain antibody was evaluated by measuring the proliferation activity to Ba/F3 cells (BaF/mpl) expressing human TPO receptor (MPL). After washing BaF/Mpl cells two times by RPMI1640 culture medium (GIBCO) containing 10% bovine fetal serum (HyClone), the cells were suspended in the culture medium at cell density of 5×10^5 cells/ml. The anti-MPL single chain antibody and human TPO (R&D Systems) was diluted with the culture medium, respectively. 50 μ l of the cell suspension and 50 μ l of the diluted antibody or human TPO were added in 96-well microplate (flat bottom) (Falcon), and cultivated in CO₂ incubator (CO₂ concentration: 5%) for 24 hours. After the incubation 10 μ l of WST-8 reagent (reagent for measuring the number of raw cells SF: Nacalai Tesque) was added and the absorbance was immediately measured at measurement wavelength of 450 nm and at reference wavelength of 620 nm using fluorescence absorbency photometer SPECTRA Fluor (TECAN). After incubating in CO₂ incubator (CO₂ concentration: 5%) for 2 hours, the absorbance at 450 nm of measurement wavelength and 620 nm of reference wavelength was again measured using SPECTRA Fluor. Since WST-8 reagent developed the color reaction depending upon the number of live cells at wavelength of 450 nm, the proliferation activity of BaF/Mpl was evaluated based on the change of absorbance in 2 hours.

[0321] The results of the agonist activity to MPL measured by using culture supernatants of COS-7 cells expressing

various 12B5 antibody molecules showed as illustrated in FIG. 51 that 12B5IgG having bivalent antigen-binding site increased the absorbance in concentration-dependent manner and had TPO-like agonist activity (ED50; 29 nM), while the agonist activity of 12B5Fab having monovalent antigen-binding site was very weak (ED50; 34,724 nM). On the contrary the single chain Fv (sc12B5) having monovalent antigen-binding site like Fab showed strong agonist activity at a level that ED50 was 75 nM. However it had been known that variable regions of H chain and L chain of the single chain Fv were associated through non-covalent bond and, therefore, each variable region was dissociated in a solution and could be associated with variable region of other molecule to form multimers like dimers. When the molecular weight of sc12B5 purified by gel filtration was measured, it was confirmed that there were molecules recognized to be monomer and dimer (see FIG. 48). Then monomer sc12B5 and dimer sc12B5 were isolated (see FIG. 50) and measured for the agonist activity to MPL. As shown in FIGS. 51 and 52, ED50 of sc12B5 monomer was 4438.7 nM, which confirmed that the agonist activity was reduced compared with the result using culture supernatant of COS-7 cells. On the contrary single chain Fv (sc12B5 dimer) having bivalent antigen-binding site showed about 400-fold stronger agonist activity (ED50; 10.1 nM) compared with monovalent sc12B5. Furthermore, the bivalent single chain Fv showed the agonist activity equivalent to or higher than the agonist activity of human TPO and 12B5IgG.

EXPLANATION OF DRAWINGS

[0322] FIG. 1 shows the result of flow cytometry, illustrating that human IgG antibody does not bind to L1210 cells expressing human IAP (hIAP/L1210).

[0323] FIG. 2 shows the result of flow cytometry, illustrating that the chimera MABL-1 antibody specifically binds to L1210 cells expressing human IAP (hIAP/L1210).

[0324] FIG. 3 shows the result of flow cytometry, illustrating that the chimera MABL-2 antibody specifically binds to L1210 cells expressing human IAP (hIAP/L1210).

[0325] FIG. 4 schematically illustrates the process for producing the single chain Fv according to the present invention.

[0326] FIG. 5 illustrates a structure of an expression plasmid which can be used to express a DNA encoding the single chain Fv of the invention in *E. coli*.

[0327] FIG. 6 illustrates a structure of an expression plasmid which is used to express a DNA encoding the single chain Fv of the invention in mammalian cells.

[0328] FIG. 7 shows a photograph showing the result of western blotting in Example 5.4. From the left, a molecular weight marker (which indicates 97.4, 66, 45, 31, 21.5 and 14.5 kDa from the top), the culture supernatant of pCHO1-introduced COS7 cells and the culture supernatant of pCHOM2-introduced COS7 cells. It illustrates that the reconstructed single chain Fv of the antibody MABL-2 (arrow) is contained in the culture supernatant of the pCHOM2-introduced cells.

[0329] FIG. 8 shows the result of flow cytometry, illustrating that an antibody in the culture supernatant of pCHO1/COS7 cell as a control does not bind to pCOS1/L1210 cell as a control.

[0330] FIG. 9 shows the result of flow cytometry, illustrating that an antibody in the culture supernatant of MABL2-scFv/COS7 cells does not bind to pCOS1/L1210 cells as a control.

[0331] FIG. 10 shows the result of flow cytometry, illustrating that an antibody in the culture supernatant of pCOS1/COS7 cells as a control does not bind to hIAP/L1210 cells.

[0332] FIG. 11 shows the result of flow cytometry, illustrating that an antibody in the culture supernatant of MABL2-scFv/COS7 cells specifically binds to hIAP/L1210 cells.

[0333] FIG. 12 shows the result of the competitive ELISA in Example 5.6, wherein the binding activity of the single chain Fv of the invention (MABL2-scFv) to the antigen is demonstrated in terms of the inhibition of binding of the mouse monoclonal antibody MABL-2 to the antigen as an index, in comparison with the culture supernatant of pCHO1/COS7 cells as a control.

[0334] FIG. 13 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of pCHO1/COS7 cells as a control does not induce the apoptosis of pCOS1/L1210 cells as a control.

[0335] FIG. 14 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of MABL2-scFv/COS7 cells does not induce apoptosis of pCOS1/L1210 cells as a control.

[0336] FIG. 15 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of pCHO1/COS7 cells as a control does not induce apoptosis of hIAP/L1210 cells.

[0337] FIG. 16 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of MABL2-scFv/COS7 cells specifically induces apoptosis of hIAP/L1210 cells.

[0338] FIG. 17 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of pCHO1/COS7 cells as a control does not induce apoptosis of CCRF-CEM cells (at 50% of the final concentration).

[0339] FIG. 18 shows the results of the apoptosis-inducing effect in Example 5.7, illustrating that the antibody in the culture supernatant of MABL2-scFv/COS7 cells specifically induces apoptosis of CCRF-CEM cells (at 50% of the final concentration).

[0340] FIG. 19 shows the chromatogram obtained in the purification of the single chain Fv derived from the antibody MABL-2 produced by the CHO cells in Example 5.9, illustrating that fraction A and fraction B were obtained as the major peaks when the fraction from Blue-sepharose column was purified with hydroxyapatite column.

[0341] FIG. 20 shows the results of purification by gel filtration of fraction A and fraction B obtained in Example 5.9-(2), illustrating that the major peaks (AI and BI, respectively) were eluted from fraction A at approximately 36 kD of the apparent molecular weight and from fraction B at approximately 76 kD.

[0342] FIG. 21 is the analysis on SDS-PAGE of the fractions obtained in the purification of the single chain Fv

derived from the antibody MABL-2 produced by the CHO cells in Example 5.9, illustrating that a single band of approximately 35 kD of molecular weight was observed in both fractions.

[0343] FIG. 22 shows the results of analysis of fractions AI and BI obtained by gel filtration in the purification of the single chain Fv derived from the antibody MABL-2 produced by the CHO cells, wherein fraction AI comprises monomer and fraction BI comprises dimer.

[0344] FIG. 23 illustrates a structure of an expression plasmid which can be used to express a DNA encoding the single chain Fv of the invention in *E. coli*.

[0345] FIG. 24 shows the results of purification on the gel filtration column of crude products of the single chain Fv polypeptide derived from the antibody MABL-2 produced by *E. coli* obtained in Example 5.12, wherein each peak indicates monomer or dimer, respectively, of the single chain Fv produced by *E. coli*.

[0346] FIG. 25 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that mouse IgG antibody as a control does not induce apoptosis of hIAP/L1210 cells (the final concentration of 3 $\mu\text{g/ml}$).

[0347] FIG. 26 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that the dimer of MABL2-scFv produced by the CHO cells remarkably induces apoptosis of hIAP/L1210 cells (the final concentration of 3 $\mu\text{g/ml}$).

[0348] FIG. 27 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that the dimer of MABL2-scFv produced by *E. coli* remarkably induces apoptosis of hIAP/L1210 cells (the final concentration of 3 $\mu\text{g/ml}$).

[0349] FIG. 28 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that apoptosis induction to hIAP/L1210 cells by the MABL2-scFv monomer produced by the CHO cells is the same level as that of the control (the final concentration of 3 $\mu\text{g/ml}$).

[0350] FIG. 29 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that apoptosis induction to hIAP/L1210 cells of the MABL2-scFv monomer produced by *E. coli* is the same level as that of control (the final concentration of 3 $\mu\text{g/ml}$).

[0351] FIG. 30 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that mouse IgG antibody used as a control does not induce apoptosis of hIAP/L1210 cells even when anti-FLAG antibody is added (the final concentration of 3 $\mu\text{g/ml}$).

[0352] FIG. 31 shows the results of the apoptosis-inducing effect in Example 5.13, illustrating that MABL2-scFv monomer produced by the CHO cells remarkably induces apoptosis of hIAP/L1210 cells when anti-FLAG antibody is added (the final concentration of 3 $\mu\text{g/ml}$).

[0353] FIG. 32 shows the results of quantitative measurement of human IgG in the serum of a human myeloma cell line KPMM2-transplanted mouse, indicating amounts of human IgG produced by the human myeloma cells in the mouse. It illustrates that the dimer of scFv/CHO remarkably inhibited growth of the KPMM2 cells.

[0354] FIG. 33 shows the survival time of the mouse after the transplantation of tumor, illustrating that the scFv/CHO dimer-administered group elongated remarkably the survival time.

[0355] FIG. 34 illustrates a structure of an expression plasmid which expresses a modified antibody [sc(Fv)₂] comprising two H chain V regions and two L chain V regions derived from the antibody MABL-2.

[0356] FIG. 35 illustrates a structure of a plasmid which expresses a scFv (HL type) wherein the V regions are linked in the manner of [H chain]-[L chain] without a peptide linker.

[0357] FIG. 36 illustrates a structure of the HL-type polypeptide and amino acid sequences of peptide linkers.

[0358] FIG. 37 illustrates a structure of a plasmid which expresses a scFv (LH type) wherein the V regions are linked in the manner of [L chain]-[H chain] without a peptide linker.

[0359] FIG. 38 illustrates a structure of the LH-type polypeptide and amino acid sequences of peptide linkers.

[0360] FIG. 39 shows the results of the western blotting in Example 6.4, illustrating that the modified antibody sc(FV)₂ comprising two H chain V regions and two L chain V regions, and the MABL2-scFv having peptide linkers with different length are expressed.

[0361] FIGS. 40a and 40b show the results of flow cytometry using the culture supernatant of COS7 cells prepared in Example 6.3 (1), illustrating that the MABL2-scFv and sc(Fv)₂ having peptide linkers with different length have high affinities against human IAP.

[0362] FIG. 41 shows the results of the apoptosis-inducing effect in Example 6.6, illustrating that the scFv <HL3, 4, 6, 7, LH3, 4, 6 and 7> and the sc(Fv)₂ remarkably induce cell death of hIAP/L1210 cells.

[0363] FIG. 42 shows the results of the evaluation of antigen binding capacity in Example 6.10, illustrating that the dimer of scFv <HL5> and sc(FV)₂ have high affinities against human IAP.

[0364] FIG. 43 shows the results of the in vitro apoptosis-inducing effect in Example 6.11, illustrating that the dimer of scFv <HL5> and the sc(Fv)₂ induce apoptosis of hIAP/L1210 cells and CCRF-CEM cells in concentration-dependent manner.

[0365] FIG. 44 shows the results of the quantitative measurement of M protein produced by a human myeloma cell line KPMM2 in the serum of the human myeloma cell-transplanted mouse. It illustrates that the dimer of scFv <HL5> and the sc(Fv)₂ remarkably inhibited growth of the KPMM2 cells.

[0366] FIG. 45 shows the survival time (days) of mice after the transplantation of tumor, illustrating that the survival time of the scFv <HL5> administrated-group was remarkably prolonged.

[0367] FIG. 46 shows the survival time (days) of mice after the transplantation of tumor, illustrating that the survival time of the sc(Fv)₂ administrated-group was remarkably prolonged.

[0368] FIG. 47 is a scheme showing the method for constructing DNA fragment encoding the reconstructed 12B5 single chain Fv containing the linker sequence consisting of 15 amino acids and the structure thereof.

[0369] FIG. 48 shows the purification result of each 12B5 single chain Fv by gel filtration obtained in Example 7.5 (1), illustrating that sc12B5 was divided into two peaks (fractions A and B).

[0370] FIG. 49 shows the analytical result of each fraction A and B by SDS-PAGE performed in Example 7.5 (2).

[0371] FIG. 50 shows the analytical result of each fraction A and B by Superdex200 column performed in Example 7.5 (2), illustrating that the major peak of fraction A was eluted at an apparent molecular weight of about 44 kD shown in (a) and that the major peak of fraction B was eluted at an apparent molecular weight of about 22 kD shown in (b).

[0372] FIG. 51 shows the measurement result of the TPO-like agonist activity of sc12B5 and antibody 12B5 (IgG, Fab), illustrating that 12B5IgG and monovalent single chain Fv (sc12B5) showed TPO-like agonist activity in concentration-dependent manner.

[0373] FIG. 52 shows the measurement result of TOP-like agonist activity of sc12B5 monomer and dimer, illustrating that single chain Fv (sc12B5 dimer) having bivalent antigen-binding site had agonist activity about 400-fold higher than monovalent sc12B5 and that the efficacy is equivalent to or higher than human TPO.

INDUSTRIAL APPLICABILITY

[0374] The modified antibodies of the invention have an agonist action capable of transducing a signal into cells by crosslinking a cell surface molecule(s) and are advantageous in that the permeability to tissues and tumors is high due to the lowered molecular size compared with antibody molecule (whole IgG). The modified antibodies have remarkably higher activity compared with the original antibodies, which is attributable to that the modified antibodies are in a shape closer to a ligand compared with original antibodies. Therefore the modified antibodies can be used as signal-transducing agonists. The modification of antibody molecule results in the reduction of side effects caused by intercellular crosslinking and provides novel medicines inducing only required action by crosslinking a cell surface molecule(s). Medical preparations containing as active ingredient the modified antibody of the invention are useful as preventives and/or remedies for cancers, inflammation, hormone disorders and blood diseases, for example, leukemia, malignant lymphoma, aplastic anemia, myelodysplasia syndrome and polycythemia vera.

 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 138

<210> SEQ ID NO 1
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 1

ccatcctaatacgcactcactatagggc 27

<210> SEQ ID NO 2
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 2

ggatcccgggtggatgggtgggaagatg 27

<210> SEQ ID NO 3
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 3

ggatcccgggccaagtggatagacagatg 28

<210> SEQ ID NO 4
 <211> LENGTH: 26
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 4

ggatcccgggagtgatagaccgatg 26

<210> SEQ ID NO 5
 <211> LENGTH: 394
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(393)
 <223> OTHER INFORMATION: pGEM-M1L. 1-57; signal peptide, 58-394; mature peptide

<400> SEQUENCE: 5

atg aag ttg cct gtt agg ctg ttg gtg ctg atg ttc tgg att cct gcg 48
 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 1 5 10 15

tcc agc agt gat gtt gtg atg acc caa act cca ctc tcc ctg cct gtc 96
 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 20 25 30

agt ctt gga gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt 144
 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 35 40 45

-continued

```

cta cac agt aaa gga aac acc tat tta caa tgg tac cta cag aag cca    192
Leu His Ser Lys Gly Asn Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro
   50                               55                               60

ggc cag tct cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct    240
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
   65                               70                               75                               80

ggg gtc cca gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca    288
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                               85                               90                               95

ctc aag atc agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc    336
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
                               100                               105                               110

tct caa agt aca cat gtt ccg tac acg tcc gga ggg ggg acc aag ctg    384
Ser Gln Ser Thr His Val Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu
   115                               120                               125

gaa ata aaa c    394
Glu Ile Lys
   130

<210> SEQ ID NO 6
<211> LENGTH: 409
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(408)
<223> OTHER INFORMATION: pGEM-M1H. 1-57; signal peptide, 58-408; mature
      peptide

<400> SEQUENCE: 6

atg gaa tgg agc tgg ata ttt ctc ttc ctc ctg tca gga act gca ggt    48
Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
   1                               5                               10                               15

gtc cac tcc cag gtc cag ctg cag cag tct gga cct gac ctg gta aag    96
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Asp Leu Val Lys
                               20                               25                               30

cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc    144
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
   35                               40                               45

gtt aac cat gtt atg cac tgg gtg aag cag aag cca ggg cag ggc ctt    192
Val Asn His Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
   50                               55                               60

gag tgg att gga tat att tat cct tac aat gat ggt act aag tac aat    240
Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn
   65                               70                               75                               80

gag aag ttc aag ggc aag gcc aca ctg act tca gag aaa tcc tcc agc    288
Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Glu Lys Ser Ser Ser
   85                               90                               95

gca gcc tac atg gag ctc agc agc ctg gcc tct gag gac tct gcg gtc    336
Ala Ala Tyr Met Glu Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
   100                               105                               110

tac tac tgt gca aga ggg ggt tac tat agt tac gac gac tgg ggc caa    384
Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Ser Tyr Asp Asp Trp Gly Gln
   115                               120                               125

ggc acc act ctc aca gtc tcc tca g    409
Gly Thr Thr Leu Thr Val Ser Ser
   130                               135

```

<210> SEQ ID NO 7

-continued

```

<211> LENGTH: 394
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(393)
<223> OTHER INFORMATION: pGEM-M2L. 1-57; signal peptide, 58-393; mature
peptide

<400> SEQUENCE: 7

atg aag ttg cct gtt agg ctg ttg gtg ctg atg ttc tgg att cct ggt      48
Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Gly
  1             5             10             15

tcc agc agt gat gtt gtg atg acc caa agt cca ctc tcc ctg cct gtc      96
Ser Ser Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
             20             25             30

agt ctt gga gat caa gcc tcc atc tct tgc aga tca agt cag agc ctt     144
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
             35             40             45

gtg cac agt aat gga aag acc tat tta cat tgg tac ctg cag aag cca     192
Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
             50             55             60

ggc cag tct cca aaa ctc ctg atc tac aaa gtt tcc aac cga ttt tct     240
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
             65             70             75             80

ggg gtc cca gac agg ttc agt ggc agt gga tca gtg aca gat ttc aca     288
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp Phe Thr
             85             90             95

ctc atg atc agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc     336
Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
             100            105            110

tct caa agt aca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg     384
Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
             115            120            125

gaa ata aaa c      394
Glu Ile Lys
             130

<210> SEQ ID NO 8
<211> LENGTH: 409
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(408)
<223> OTHER INFORMATION: pGEM-M2H. 1-57; signal peptide, 58-408; mature
peptide

<400> SEQUENCE: 8

atg gaa tgg agc tgg ata ttt ctc ttc ctc ctg tca gga act gca ggt      48
Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
  1             5             10             15

gtc cac tcc cag gtc cag ctg cag cag tct gga cct gaa ctg gta aag      96
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
             20             25             30

cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc     144
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
             35             40             45

gct aac cat gtt att cac tgg gtg aag cag aag cca ggg cag ggc ctt     192
Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
             50             55             60

```

-continued

<400> SEQUENCE: 13
catgccatgg cgcaggtcca gctgcagcag 30

<210> SEQ ID NO 14
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 14
accaccacct gaggagactg tgagagt 27

<210> SEQ ID NO 15
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 15
gtctcctcag gtggtggtgg ttcgggt 27

<210> SEQ ID NO 16
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 16
cacaacatcc gatccgccac caccga 27

<210> SEQ ID NO 17
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 17
ggcggatcgg atgttgtgat gacccaa 27

<210> SEQ ID NO 18
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 18
ccggaattct cattatttat cgtcatcgtc tttgtagtct tttatttcca gcttggt 57

<210> SEQ ID NO 19
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
DNA encoding a linker amino acid sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(45)

-continued

<400> SEQUENCE: 19

```

ggt ggt ggt ggt tcg ggt ggt ggt ggt tcg ggt ggt ggc gga tcg      45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
  1             5             10             15

```

<210> SEQ ID NO 20

<211> LENGTH: 828

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(822)

<223> OTHER INFORMATION: pscM1. MABL1-scFv

<400> SEQUENCE: 20

```

atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc gct      48
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1             5             10             15

```

```

gcc caa cca gcc atg gcg cag gtc cag ctg cag cag tct gga cct gac      96
Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln Ser Gly Pro Asp
             20             25             30

```

```

ctg gta aag cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga      144
Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly
             35             40             45

```

```

tac acc ttc gtt aac cat gtt atg cac tgg gtg aag cag aag cca ggg      192
Tyr Thr Phe Val Asn His Val Met His Trp Val Lys Gln Lys Pro Gly
             50             55             60

```

```

cag ggc ctt gag tgg att gga tat att tat cct tac aat gat ggt act      240
Gln Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr
             65             70             75             80

```

```

aag tac aat gag aag ttc aag ggc aag gcc aca ctg act tca gag aaa      288
Lys Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Glu Lys
             85             90             95

```

```

tcc tcc agc gca gcc tac atg gag ctc agc agc ctg gcc tct gag gac      336
Ser Ser Ser Ala Ala Tyr Met Glu Leu Ser Ser Leu Ala Ser Glu Asp
             100             105             110

```

```

tct gcg gtc tac tac tgt gca aga ggg ggt tac tat agt tac gac gac      384
Ser Ala Val Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Ser Tyr Asp Asp
             115             120             125

```

```

tgg ggc caa ggc acc act ctc aca gtc tcc tca ggt ggt ggt ggt tcg      432
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
             130             135             140

```

```

ggt ggt ggt ggt tcg ggt ggt ggc gga tcg gat gtt gtg atg acc caa      480
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln
             145             150             155             160

```

```

act cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct      528
Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
             165             170             175

```

```

tgc aga tct agt cag agc ctt cta cac agt aaa gga aac acc tat tta      576
Cys Arg Ser Ser Gln Ser Leu Leu His Ser Lys Gly Asn Thr Tyr Leu
             180             185             190

```

```

caa tgg tac cta cag aag cca ggc cag tct cca aag ctc ctg atc tac      624
Gln Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
             195             200             205

```

```

aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt      672
Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
             210             215             220

```

```

gga tca ggg aca gat ttc aca ctc aag atc agc aga gtg gag gct gag      720
Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu

```

-continued

225	230	235	240	
gat ctg gga gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg				768
Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr	245	250	255	
tcc gga ggg ggg acc aag ctg gaa ata aaa gac tac aaa gac gat gac				816
Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp Asp	260	265	270	
gat aaa taatga				828
Asp Lys				
<210> SEQ ID NO 21				
<211> LENGTH: 31				
<212> TYPE: DNA				
<213> ORGANISM: Artificial Sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer				
<400> SEQUENCE: 21				
acgcgctcgac tcccaggtcc agctgcagca g				31
<210> SEQ ID NO 22				
<211> LENGTH: 18				
<212> TYPE: DNA				
<213> ORGANISM: Artificial Sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer				
<400> SEQUENCE: 22				
gaaggtgtat ccagaagc				18
<210> SEQ ID NO 23				
<211> LENGTH: 819				
<212> TYPE: DNA				
<213> ORGANISM: Mus musculus				
<220> FEATURE:				
<221> NAME/KEY: CDS				
<222> LOCATION: (1)..(813)				
<223> OTHER INFORMATION: pCHOM1. MABL1-scFv				
<400> SEQUENCE: 23				
atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt				48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly	1	5	10	15
gtc gac tcc cag gtc cag ctg cag cag tct gga cct gac ctg gta aag				96
Val Asp Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Asp Leu Val Lys	20	25	30	
cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc				144
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe	35	40	45	
gtt aac cat gtt atg cac tgg gtg aag cag aag cca ggg cag ggc ctt				192
Val Asn His Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu	50	55	60	
gag tgg att gga tat att tat cct tac aat gat ggt act aag tac aat				240
Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn	65	70	75	80
gag aag ttc aag ggc aag gcc aca ctg act tca gag aaa tcc tcc agc				288
Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Glu Lys Ser Ser Ser	85	90	95	
gca gcc tac atg gag ctc agc agc ctg gcc tct gag gac tct gcg gtc				336
Ala Ala Tyr Met Glu Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val	100	105	110	

-continued

tac tac tgt gca aga ggg ggt tac tat agt tac gac gac tgg ggc caa Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Ser Tyr Asp Asp Trp Gly Gln 115 120 125	384
ggc acc act ctc aca gtc tcc tca ggt ggt ggt ggt tcg ggt ggt ggt Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly 130 135 140	432
ggt tcg ggt ggt ggc gga tcg gat gtt gtg atg acc caa act cca ctc Gly Ser Gly Gly Gly Ser Asp Val Val Met Thr Gln Thr Pro Leu 145 150 155 160	480
tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga tct Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser 165 170 175	528
agt cag agc ctt cta cac agt aaa gga aac acc tat tta caa tgg tac Ser Gln Ser Leu Leu His Ser Lys Gly Asn Thr Tyr Leu Gln Trp Tyr 180 185 190	576
cta cag aag cca ggc cag tct cca aag ctc ctg atc tac aaa gtt tcc Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser 195 200 205	624
aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca ggg Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly 210 215 220	672
aca gat ttc aca ctc aag atc agc aga gtg gag gct gag gat ctg gga Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly 225 230 235 240	720
gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg tcc gga ggg Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Ser Gly Gly 245 250 255	768
ggg acc aag ctg gaa ata aaa gac tac aaa gac gat gac gat aaa Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp Asp Asp Lys 260 265 270	813
taatga	819
 <210> SEQ ID NO 24 <211> LENGTH: 828 <212> TYPE: DNA <213> ORGANISM: Mus musculus <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(822) <223> OTHER INFORMATION: pscM2. MABL2-scFv <400> SEQUENCE: 24	
atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc gct Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala 1 5 10 15	48
gcc caa cca gcc atg gcg cag gtc cag ctg cag cag tct gga gct gaa Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala Glu 20 25 30	96
ctg gta aag cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly 35 40 45	144
tac acc ttc gct aac cat gtt att cac tgg gtg aag cag aag cca ggg Tyr Thr Phe Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly 50 55 60	192
cag ggc ctt gag tgg att gga tat att tat cct tac aat gat ggt act Gln Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr 65 70 75 80	240
aag tat aat gag aag ttc aag gac aag gcc act ctg act tca gac aaa	288

-continued

Lys	Tyr	Asn	Glu	Lys	Phe	Lys	Asp	Lys	Ala	Thr	Leu	Thr	Ser	Asp	Lys	
				85					90						95	
tcc	tcc	acc	aca	gcc	tac	atg	gac	ctc	agc	agc	ctg	gcc	tct	gag	gac	336
Ser	Ser	Thr	Thr	Ala	Tyr	Met	Asp	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	
			100					105						110		
tct	gcg	gtc	tat	tac	tgt	gca	aga	ggg	ggg	tac	tat	act	tac	gac	gac	384
Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Gly	Tyr	Tyr	Thr	Tyr	Asp	Asp	
			115					120						125		
tgg	ggc	caa	ggc	acc	act	ctc	aca	gtc	tcc	tca	ggt	ggg	ggg	ggt	tcg	432
Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	
			130				135							140		
ggg	ggg	ggg	ggg	tcg	ggg	ggg	ggc	gga	tcg	gat	ggt	gtg	atg	acc	caa	480
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Val	Val	Met	Thr	Gln	
			145			150				155					160	
agt	cca	ctc	tcc	ctg	cct	gtc	agt	ctt	gga	gat	caa	gcc	tcc	atc	tct	528
Ser	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly	Asp	Gln	Ala	Ser	Ile	Ser	
			165						170					175		
tgc	aga	tca	agt	cag	agc	ctt	gtg	cac	agt	aat	gga	aag	acc	tat	tta	576
Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser	Asn	Gly	Lys	Thr	Tyr	Leu	
			180					185						190		
cat	tgg	tac	ctg	cag	aag	cca	ggc	cag	tct	cca	aaa	ctc	ctg	atc	tac	624
His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	
			195				200							205		
aaa	ggt	tcc	aac	cga	ttt	tct	ggg	gtc	cca	gac	agg	ttc	agt	ggc	agt	672
Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	
			210			215								220		
gga	tca	gtg	aca	gat	ttc	aca	ctc	atg	atc	agc	aga	gtg	gag	gct	gag	720
Gly	Ser	Val	Thr	Asp	Phe	Thr	Leu	Met	Ile	Ser	Arg	Val	Glu	Ala	Glu	
			225			230				235					240	
gat	ctg	gga	ggt	tat	ttc	tgc	tct	caa	agt	aca	cat	ggt	ccg	tac	acg	768
Asp	Leu	Gly	Val	Tyr	Phe	Cys	Ser	Gln	Ser	Thr	His	Val	Pro	Tyr	Thr	
			245							250					255	
ttc	gga	ggg	ggg	acc	aag	ctg	gaa	ata	aaa	gac	tac	aaa	gac	gat	gac	816
Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Asp	Tyr	Lys	Asp	Asp	Asp	
			260				265							270		
gat	aaa	taatga														828
Asp	Lys															
<210> SEQ ID NO 25																
<211> LENGTH: 819																
<212> TYPE: DNA																
<213> ORGANISM: Mus musculus																
<220> FEATURE:																
<221> NAME/KEY: CDS																
<222> LOCATION: (1)..(813)																
<223> OTHER INFORMATION: pCHOM2. MABL2-scFv																
<400> SEQUENCE: 25																
atg	gga	tgg	agc	tgt	atc	atc	ctc	ttc	ttg	gta	gca	aca	gct	aca	ggg	48
Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	Gly	
		1				5				10					15	
gtc	gac	tcc	cag	gtc	cag	ctg	cag	cag	tct	gga	cct	gaa	ctg	gta	aag	96
Val	Asp	Ser	Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	
			20						25					30		
cct	ggg	gct	tca	gtg	aag	atg	tcc	tgc	aag	gct	tct	gga	tac	acc	ttc	144
Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	
			35				40						45			
gct	aac	cat	ggt	att	cac	tgg	gtg	aag	cag	aag	cca	ggg	cag	ggc	ctt	192
Ala	Asn	His	Val	Ile	His	Trp	Val	Lys	Gln	Lys	Pro	Gly	Gln	Gly	Leu	

-continued

50	55	60	
gag tgg att gga tat att tat cct tac aat gat ggt act aag tat aat			240
Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn			
65	70	75	80
gag aag ttc aag gac aag gcc act ctg act tca gac aaa tcc tcc acc			288
Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr			
	85	90	95
aca gcc tac atg gac ctc agc agc ctg gcc tct gag gac tct gcg gtc			336
Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val			
	100	105	110
tat tac tgt gca aga ggg ggt tac tat act tac gac gac tgg ggc caa			384
Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln			
	115	120	125
ggc acc act ctc aca gtc tcc tca ggt ggt ggt ggt tcc ggt ggt ggt			432
Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly			
	130	135	140
ggt tcc ggt ggt ggc gga tcc gat gtt gtg atg acc caa agt cca ctc			480
Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Ser Pro Leu			
	145	150	155
tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga tca			528
Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser			
	165	170	175
agt cag agc ctt gtg cac agt aat gga aag acc tat tta cat tgg tac			576
Ser Gln Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr			
	180	185	190
ctg cag aag cca ggc cag tct cca aaa ctc ctg atc tac aaa gtt tcc			624
Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser			
	195	200	205
aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca gtg			672
Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val			
	210	215	220
aca gat ttc aca ctc atg atc agc aga gtg gag gct gag gat ctg gga			720
Thr Asp Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly			
	225	230	235
ggt tat ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga ggg			768
Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly			
	245	250	255
ggg acc aag ctg gaa ata aaa gac tac aaa gac gat gac gat aaa			813
Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp Asp Asp Lys			
	260	265	270
taatga			819
<p><210> SEQ ID NO 26 <211> LENGTH: 456 <212> TYPE: DNA <213> ORGANISM: Mus musculus <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(450) <223> OTHER INFORMATION: pCHO-shIAP. Soluble human IAP</p>			
<p><400> SEQUENCE: 26</p>			
atg tgg ccc ctg gta gcg gcg ctg ttg ctg ggc tcc gcg tgc tgc gga			48
Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly			
1	5	10	15
tca gct cag cta cta ttt aat aaa aca aaa tct gta gaa ttc acg ttt			96
Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe			
	20	25	30

-continued

tgt aat gac act gtc gtc att cca tgc ttt gtt act aat atg gag gca Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala 35 40 45	144
caa aac act act gaa gta tac gta aag tgg aaa ttt aaa gga aga gat Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp 50 55 60	192
att tac acc ttt gat gga gct cta aac aag tcc act gtc ccc act gac Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Val Pro Thr Asp 65 70 75 80	240
ttt agt agt gca aaa att gaa gtc tca caa tta cta aaa gga gat gcc Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala 85 90 95	288
tct ttg aag atg gat aag agt gat gct gtc tca cac aca gga aac tac Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr 100 105 110	336
act tgt gaa gta aca gaa tta acc aga gaa ggt gaa acg atc atc gag Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu 115 120 125	384
cta aaa tat cgt gtt gtt tca tgg ttt tct cca aat gaa aat gac tac Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Asn Glu Asn Asp Tyr 130 135 140	432
aag gac gac gat gac aag tgatag Lys Asp Asp Asp Asp Lys 145 150	456
<210> SEQ ID NO 27	
<211> LENGTH: 46	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer	
<400> SEQUENCE: 27	
ggaattccat atgcaagtgc aacttcaaca gtctggacct gaactg	46
<210> SEQ ID NO 28	
<211> LENGTH: 31	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer	
<400> SEQUENCE: 28	
ggaattctca ttattttatt tccagcttgg t	31
<210> SEQ ID NO 29	
<211> LENGTH: 741	
<212> TYPE: DNA	
<213> ORGANISM: Mus musculus	
<220> FEATURE:	
<221> NAME/KEY: CDS	
<222> LOCATION: (1)..(735)	
<223> OTHER INFORMATION: pscM2DEm02. MABL2-scFv	
<400> SEQUENCE: 29	
atg caa gtg caa ctt caa cag tct gga cct gaa ctg gta aag cct ggg Met Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly 1 5 10 15	48
gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc gct aac Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn 20 25 30	96

-continued

cat gtt att cac tgg gtg aag cag aag cca ggg cag ggc ctt gag tgg	144
His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp	
35 40 45	
att gga tat att tat cct tac aat gat ggt act aag tat aat gag aag	192
Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys	
50 55 60	
ttc aag gac aag gcc act ctg act tca gac aaa tcc tcc acc aca gcc	240
Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr Thr Ala	
65 70 75 80	
tac atg gac ctc agc agc ctg gcc tct gag gac tct gcg gtc tat tac	288
Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr	
85 90 95	
tgt gca aga ggg ggt tac tat act tac gac gac tgg ggc caa ggc acc	336
Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln Gly Thr	
100 105 110	
act ctc aca gtc tcc tca ggt ggt ggt ggt tcg ggt ggt ggt tcg	384
Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser	
115 120 125	
ggt ggt ggc gga tcg gat gtt gtg atg acc caa agt cca ctc tcc ctg	432
Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu	
130 135 140	
cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga tca agt cag	480
Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln	
145 150 155 160	
agc ctt gtg cac agt aat gga aag acc tat tta cat tgg tac ctg cag	528
Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln	
165 170 175	
aag cca ggc cag tct cca aaa ctc ctg atc tac aaa gtt tcc aac cga	576
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg	
180 185 190	
ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca gtg aca gat	624
Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp	
195 200 205	
ttc aca ctc atg atc agc aga gtg gag gct gag gat ctg gga gtt tat	672
Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr	
210 215 220	
ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga ggg ggg acc	720
Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr	
225 230 235 240	
aag ctg gaa ata aaa taatga	741
Lys Leu Glu Ile Lys	
245	

<210> SEQ ID NO 30
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 30

cagacagtgg ttcaaagt 18

<210> SEQ ID NO 31
 <211> LENGTH: 72
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

-continued

<400> SEQUENCE: 31

cgcgctcgacc gatccgccac caccggaacc accaccaccc gaaccaccac caccttttat 60
 ttccagcttg gt 72

<210> SEQ ID NO 32

<211> LENGTH: 1605

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1599)

<223> OTHER INFORMATION: pCHOM2(Fv)2. MABL2-sc(Fv)2

<400> SEQUENCE: 32

atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt 48
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

gtc gac tcc cag gtc cag ctg cag cag tct gga cct gaa ctg gta aag 96
 Val Asp Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30

cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc 144
 Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

gct aac cat gtt att cac tgg gtg aag cag aag cca ggg cag ggc ctt 192
 Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60

gag tgg att gga tat att tat cct tac aat gat ggt act aag tat aat 240
 Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn
 65 70 75 80

gag aag ttc aag gac aag gcc act ctg act tca gac aaa tcc tcc acc 288
 Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr
 85 90 95

aca gcc tac atg gac ctc agc agc ctg gcc tct gag gac tct gcg gtc 336
 Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
 100 105 110

tat tac tgt gca aga ggg ggt tac tat act tac gac gac tgg ggc caa 384
 Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln
 115 120 125

ggc acc act ctc aca gtc tcc tca ggt ggt ggt ggt tcg ggt ggt ggt 432
 Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
 130 135 140

ggt tcg ggt ggt ggc gga tcg gat gtt gtg atg acc caa agt cca ctc 480
 Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Ser Pro Leu
 145 150 155 160

tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga tca 528
 Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser
 165 170 175

agt cag agc ctt gtg cac agt aat gga aag acc tat tta cat tgg tac 576
 Ser Gln Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr
 180 185 190

ctg cag aag cca ggc cag tct cca aaa ctc ctg atc tac aaa gtt tcc 624
 Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
 195 200 205

aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca gtg 672
 Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val
 210 215 220

aca gat ttc aca ctc atg atc agc aga gtg gag gct gag gat ctg gga 720
 Thr Asp Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly

-continued

225	230	235	240	
ggt tat ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga ggg Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly	245	250	255	768
ggg acc aag ctg gaa ata aaa ggt ggt ggt ggt tcg ggt ggt ggt ggt Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly	260	265	270	816
tcg ggt ggt ggc gga tcg gtc gac tcc cag gtc cag ctg cag cag tct Ser Gly Gly Gly Gly Ser Val Asp Ser Gln Val Gln Leu Gln Gln Ser	275	280	285	864
gga cct gaa ctg gta aag cct ggg gct tca gtg aag atg tcc tgc aag Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys	290	295	300	912
gct tct gga tac acc ttc gct aac cat gtt att cac tgg gtg aag cag Ala Ser Gly Tyr Thr Phe Ala Asn His Val Ile His Trp Val Lys Gln	305	310	315	960
aag cca ggg cag ggc ctt gag tgg att gga tat att tat cct tac aat Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn	325	330	335	1008
gat ggt act aag tat aat gag aag ttc aag gac aag gcc act ctg act Asp Gly Thr Lys Tyr Asn Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr	340	345	350	1056
tca gac aaa tcc tcc acc aca gcc tac atg gac ctc agc agc ctg gcc Ser Asp Lys Ser Ser Thr Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala	355	360	365	1104
tct gag gac tct cgg gtc tat tac tgt gca aga ggg ggt tac tat act Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr	370	375	380	1152
tac gac gac tgg ggc caa ggc acc act ctc aca gtc tcc tca ggt ggt Tyr Asp Asp Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly	385	390	395	1200
ggg ggt tcg ggt ggt ggt ggt tcg ggt ggt ggc gga tcg gat gtt gtg Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val	405	410	415	1248
atg acc caa agt cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc Met Thr Gln Ser Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala	420	425	430	1296
tcc atc tct tgc aga tca agt cag agc ctt gtg cac agt aat gga aag Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Lys	435	440	445	1344
acc tat tta cat tgg tac ctg cag aag cca ggc cag tct cca aaa ctc Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu	450	455	460	1392
ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe	465	470	475	1440
agt ggc agt gga tca gtg aca gat ttc aca ctc atg atc agc aga gtg Ser Gly Ser Gly Ser Val Thr Asp Phe Thr Leu Met Ile Ser Arg Val	485	490	495	1488
gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt aca cat gtt Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val	500	505	510	1536
ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa gac tac aaa Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys	515	520	525	1584
gac gat gac gat aaa taatga Asp Asp Asp Asp Lys				1605

-continued

530

<210> SEQ ID NO 33
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 33

tgaggaattc ccaccatggg atg 23

<210> SEQ ID NO 34
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 34

cacgacgtca ctcgagactg tgagagtggg gccttgcccc 40

<210> SEQ ID NO 35
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 35

agtctcgagt gacgtcgtga tgaccctaaag tccactctcc 40

<210> SEQ ID NO 36
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 36

gactggatcc tcattattta tcgtcatcgt c 31

<210> SEQ ID NO 37
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 37

cgcgtaatac gactcactat ag 22

<210> SEQ ID NO 38
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 38

gcaattggac ctgttttatc tcgagcttgg tccccctcc gaacgt 46

<210> SEQ ID NO 39

-continued

<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 39

gctcgagata aaacaggtcc aattgcagca gtctggacct gaact 45

<210> SEQ ID NO 40
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 40

gactgcatcc tcattattha tcgtcatcgt cttttagtgc tgaggagact gtgagagtgg 60

<210> SEQ ID NO 41
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 41

gactgaattc ccacocatgaa gttgctgtt ag 32

<210> SEQ ID NO 42
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 42

cagtctcgag tgggtggtcc gacgtcgtga tgacccaaag 40

<210> SEQ ID NO 43
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 43

cagtctcgag tgggtggtgt tccgacgtcg tgatgaccca aag 43

<210> SEQ ID NO 44
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 44

cagtctcgag tgggtggtgt gttccgacg tcgtgatgac ccaaag 46

<210> SEQ ID NO 45
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 45

cagtctcgag tgggtggtggt ggtggttcgc acgtcgtgat gacccaaag 49

<210> SEQ ID NO 46
 <211> LENGTH: 52
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 46

cagtctcgag tgggtggtggt ggtggtggtt ccgacgtcgt gatgacccaa ag 52

<210> SEQ ID NO 47
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 47

ggccgcacatgt tgtcacgaat 20

<210> SEQ ID NO 48
 <211> LENGTH: 780
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(768)
 <223> OTHER INFORMATION: CF2HL-0/pCOS1. MABL2-scFv<HL-0>

<400> SEQUENCE: 48

atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt 48
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

gtc gac tcc cag gtc cag ctg cag cag tct gga cct gaa ctg gta aag 96
 Val Asp Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30

cct ggg gct tca gtg aag atg tcc tgc aag gct tct gga tac acc ttc 144
 Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

gct aac cat gtt att cac tgg gtg aag cag aag cca ggg cag ggc ctt 192
 Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60

gag tgg att gga tat att tat cct tac aat gat ggt act aag tat aat 240
 Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn
 65 70 75 80

gag aag ttc aag gac aag gcc act ctg act tca gac aaa tcc tcc acc 288
 Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr
 85 90 95

aca gcc tac atg gac ctc agc agc ctg gcc tct gag gac tct gcg gtc 336
 Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
 100 105 110

tat tac tgt gca aga ggg ggt tac tat act tac gac gac tgg ggc caa 384
 Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln
 115 120 125

ggc acc act ctc aca gtc tcg agt gac gtc gtg atg acc caa agt cca 432
 Gly Thr Thr Leu Thr Val Ser Ser Asp Val Val Met Thr Gln Ser Pro

-continued

130	135	140	
ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga			480
Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg			
145	150	155	160
tca agt cag agc ctt gtg cac agt aat gga aag acc tat tta cat tgg			528
Ser Ser Gln Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp			
	165	170	175
tac ctg cag aag cca ggc cag tct cca aaa ctc ctg atc tac aaa gtt			576
Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val			
	180	185	190
tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca			624
Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser			
	195	200	205
gtg aca gat ttc aca ctc atg atc agc aga gtg gag gct gag gat ctg			672
Val Thr Asp Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu			
	210	215	220
gga gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga			720
Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly			
225	230	235	240
ggg ggg acc aag ctg gaa ata aaa gac tac aaa gac gat gac gat aaa			768
Gly Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp Asp Asp Lys			
	245	250	255
taatgaggat cc			780
<p><210> SEQ ID NO 49 <211> LENGTH: 45 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>			
<400> SEQUENCE: 49			
caagctcgag ataaaatccg gaggccaggt ccaattgcag cagtc			45
<p><210> SEQ ID NO 50 <211> LENGTH: 48 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>			
<400> SEQUENCE: 50			
caagctcgag ataaaatccg gagggtggcca ggtccaattg cagcagtc			48
<p><210> SEQ ID NO 51 <211> LENGTH: 51 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>			
<400> SEQUENCE: 51			
caagctcgag ataaaatccg gagggtgtgg ccaggtccaa ttgcagcagt c			51
<p><210> SEQ ID NO 52 <211> LENGTH: 54 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>			

-continued

<400> SEQUENCE: 52

caagctcgag ataaaaatccg gaggtggtgg tggccaggtc caattgcagc agtc 54

<210> SEQ ID NO 53

<211> LENGTH: 57

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 53

caagctcgag ataaaaatccg gaggtggtgg tgggtggccag gtccaattgc agcagtc 57

<210> SEQ ID NO 54

<211> LENGTH: 780

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(768)

<223> OTHER INFORMATION: CF2LH-0/pCOS1. MABL2-scFv<LH-0>

<400> SEQUENCE: 54

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

-continued

gag aag ttc aag gac aag gcc act ctg act tca gac aaa tcc tcc acc Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr 195 200 205	624
aca gcc tac atg gac ctc agc agc ctg gcc tct gag gac tct gcg gtc Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val 210 215 220	672
tat tac tgt gca aga ggg ggt tac tat act tac gac gac tgg ggc caa Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln 225 230 235 240	720
ggc acc act ctc aca gtc tcc tca gac tac aaa gac gat gac gat aaa Gly Thr Thr Leu Thr Val Ser Ser Asp Tyr Lys Asp Asp Asp Lys 245 250 255	768
taatgaggat cc	780
<p><210> SEQ ID NO 55 <211> LENGTH: 351 <212> TYPE: DNA <213> ORGANISM: Homo sapiens <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(351) <223> OTHER INFORMATION: 12B5HV. 1-351 peptide</p>	
<400> SEQUENCE: 55	
cag gtg cag ctg gtg cag tct ggg gga ggc ttg gtc cgg ccc ggg ggg Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Arg Pro Gly Gly 1 5 10 15	48
tcc ctg agt ctc tcc tgt gca gtc tct gga atc acc ctc agg acc tac Ser Leu Ser Leu Ser Cys Ala Val Ser Gly Ile Thr Leu Arg Thr Tyr 20 25 30	96
ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45	144
gca ggt ata tcc ttt gac gga aga agt gaa tac tat gca gac tcc gtg Ala Gly Ile Ser Phe Asp Gly Arg Ser Glu Tyr Tyr Ala Asp Ser Val 50 55 60	192
cag ggc cga ttc acc atc tcc aga gac agt tcc aag aac acc ctg tat Gln Gly Arg Phe Thr Ile Ser Arg Asp Ser Ser Lys Asn Thr Leu Tyr 65 70 75 80	240
ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95	288
gcg aga gga gca cat tat ggt ttc gat atc tgg ggc caa ggg aca atg Ala Arg Gly Ala His Tyr Gly Phe Asp Ile Trp Gly Gln Gly Thr Met 100 105 110	336
gtc acc gtc tcg agt Val Thr Val Ser Ser 115	351
<p><210> SEQ ID NO 56 <211> LENGTH: 57 <212> TYPE: DNA <213> ORGANISM: Homo sapiens <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(57) <223> OTHER INFORMATION: DNA encoding a leader sequence</p>	
<400> SEQUENCE: 56	
atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt tta aga ggt Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly	48

-continued

1	5	10	15		
gtc cag tgt				57	
Val Gln Cys					
<210> SEQ ID NO 57					
<211> LENGTH: 115					
<212> TYPE: DNA					
<213> ORGANISM: Artificial Sequence					
<220> FEATURE:					
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer					
<400> SEQUENCE: 57					
atggagtttg	ggctgagctg	ggttttcctc	gttgctcttt	taagaggtgt ccagtgtcag	60
gtgcagctgg	tgcaagtctg	gggaggcttg	gtccggcccc	gggggtccct gagtc	115
<210> SEQ ID NO 58					
<211> LENGTH: 115					
<212> TYPE: DNA					
<213> ORGANISM: Artificial Sequence					
<220> FEATURE:					
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer					
<400> SEQUENCE: 58					
aaggatatac	ctgccacca	ctccagcccc	ttgcctggag	cctggcggac ccagtgcag	60
ccgtaggtcc	tgagggatg	tccagagact	gcacaggaga	gactcaggga ccccc	115
<210> SEQ ID NO 59					
<211> LENGTH: 115					
<212> TYPE: DNA					
<213> ORGANISM: Artificial Sequence					
<220> FEATURE:					
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer					
<400> SEQUENCE: 59					
ggcaggtata	tcctttgac	gagaagtga	atactatgca	gactccgtgc agggccgatt	60
caccatctcc	agagacagt	ccaagaacac	cctgtatctg	caaatgaaca gcctg	115
<210> SEQ ID NO 60					
<211> LENGTH: 108					
<212> TYPE: DNA					
<213> ORGANISM: Artificial Sequence					
<220> FEATURE:					
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer					
<400> SEQUENCE: 60					
actcgagacg	gtgaccattg	tcctttggcc	ccagatatcg	aaaccataat gtgctcctct	60
cgcacagtaa	tacacagccg	tgtcctcggc	tctcaggctg	ttcatttg	108
<210> SEQ ID NO 61					
<211> LENGTH: 32					
<212> TYPE: DNA					
<213> ORGANISM: Artificial Sequence					
<220> FEATURE:					
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer					
<400> SEQUENCE: 61					
ttcaagcttc	caccatggag	tttgggctga	gc		32
<210> SEQ ID NO 62					
<211> LENGTH: 34					

-continued

```

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 65

aaaagatcctt tatcatgtgt gagttttgtc acaagatttg ggctcaactt tcttgtccac      60

<210> SEQ ID NO 66
<211> LENGTH: 432
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(419)
<223> OTHER INFORMATION: HEF-12B5H-g gamma. 12-419 peptide

<400> SEQUENCE: 66

aagcttccac c atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt      50
      Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu
      1             5             10

tta aga ggt gtc cag tgt cag gtg cag ctg gtg cag tct ggg gga ggc      98
Leu Arg Gly Val Gln Cys Gln Val Gln Leu Val Gln Ser Gly Gly Gly
      15             20             25

ttg gtc cgg ccc ggg ggg tcc ctg agt ctc tcc tgt gca gtc tct gga      146
Leu Val Arg Pro Gly Gly Ser Leu Ser Leu Ser Cys Ala Val Ser Gly
      30             35             40             45

atc acc ctc agg acc tac ggc atg cac tgg gtc cgc cag gct cca ggc      194
Ile Thr Leu Arg Thr Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly
      50             55             60

aag ggg ctg gag tgg gtg gca ggt ata tcc ttt gac gga aga agt gaa      242
Lys Gly Leu Glu Trp Val Ala Gly Ile Ser Phe Asp Gly Arg Ser Glu
      65             70             75

tac tat gca gac tcc gtg cag ggc cga ttc acc atc tcc aga gac agt      290
Tyr Tyr Ala Asp Ser Val Gln Gly Arg Phe Thr Ile Ser Arg Asp Ser
      80             85             90

tcc aag aac acc ctg tat ctg caa atg aac agc ctg aga gcc gag gac      338
Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
      95             100            105

acg gct gtg tat tac tgt gcg aga gga gca cat tat ggt ttc gat atc      386
Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ala His Tyr Gly Phe Asp Ile
      110            115            120            125

tgg ggc caa ggg aca atg gtc acc gtc tcg agt ggtgagtgga tcc      432
Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
      130            135

<210> SEQ ID NO 67
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(321)
<223> OTHER INFORMATION: 12B5LV. 1-321 peptide

<400> SEQUENCE: 67

gac atc cag atg acc cag tct cct tcc acc ctg tct gca tct att gga      48
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Ile Gly
      1             5             10            15

gac aga gtc acc atc acc tgc cgg gcc agc gag ggt att tat cac tgg      96
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Gly Ile Tyr His Trp
      20             25             30

```

-continued

```

ttg gcc tgg tat cag cag aag cca ggg aaa gcc cct aaa ctc ctg atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
      35              40              45

tat aag gcc tct agt tta gcc agt ggg gcc cca tca agg ttc agc ggc      192
Tyr Lys Ala Ser Ser Leu Ala Ser Gly Ala Pro Ser Arg Phe Ser Gly
      50              55              60

agt gga tct ggg aca gat ttc act ctc acc atc agc agc ctg cag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
      65              70              75              80

gat gat ttt gca act tat tac tgc caa caa tat agt aat tat ccg ctc      288
Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Leu
      85              90              95

act ttc gcc gga ggg acc aag ctg gag atc aaa      321
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
      100              105

```

```

<210> SEQ ID NO 68
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(66)
<223> OTHER INFORMATION: DNA encoding a leader sequence

```

```

<400> SEQUENCE: 68

```

```

atg gac atg agg gtc ccc gct cag ctc ctg ggg ctc ctg ctg ctc tgg      48
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
      1              5              10              15

ctc cca ggt gcc aaa tgt      66
Leu Pro Gly Ala Lys Cys
      20

```

```

<210> SEQ ID NO 69
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

```

```

<400> SEQUENCE: 69

```

```

atggacatga gggccccgc tcagctcctg gggtcctcgc tgctctggct ccaggtgcc      60
aaatgtgaca tccagatgac ccagtctcct tccaccctgt ctgcatctat      110

```

```

<210> SEQ ID NO 70
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

```

```

<400> SEQUENCE: 70

```

```

ggagtttagg ggctttccct ggcttctgct gataccaggc caaccagtga taaataccct      60
cgctggcccg gcaggtgatg gtgactctgt ctccaataga tgcagacagg      110

```

```

<210> SEQ ID NO 71
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

```

-continued

```

<400> SEQUENCE: 71
aagcccctaa actcctgata tataaggcct ctagttagc cagtggggcc ccatcaaggt    60
tcagcggcag tggatctggg acagatttca ctctcaccat cagcagcctg          110

<210> SEQ ID NO 72
<211> LENGTH: 103
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 72
tttgatctcc agcttggctc ctccgccgaa agtgagcggg taattactat attggttgca    60
gtaataagtt gcaaaatcat caggctgcag gctgctgatg gtg                    103

<210> SEQ ID NO 73
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 73
ttcaagcttc caccatggac atgagggctc cc                                32

<210> SEQ ID NO 74
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 74
tctagatcc actcacgttt gatctccagc ttggt                                35

<210> SEQ ID NO 75
<211> LENGTH: 415
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(398)
<223> OTHER INFORMATION: HEF-12B5H-g kappa. 12-398 peptide

<400> SEQUENCE: 75
aagcttcac c atg gac atg agg gtc ccc gct cag ctc ctg ggg ctc ctg    50
      Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu
      1          5          10

ctg ctc tgg ctc cca ggt gcc aaa tgt gac atc cag atg acc cag tct    98
Leu Leu Trp Leu Pro Gly Ala Lys Cys Asp Ile Gln Met Thr Gln Ser
      15          20          25

cct tcc acc ctg tct gca tct att gga gac aga gtc acc atc acc tgc    146
Pro Ser Thr Leu Ser Ala Ser Ile Gly Asp Arg Val Thr Ile Thr Cys
      30          35          40          45

cgg gcc agc gag ggt att tat cac tgg ttg gcc tgg tat cag cag aag    194
Arg Ala Ser Glu Gly Ile Tyr His Trp Leu Ala Trp Tyr Gln Gln Lys
      50          55          60

cca ggg aaa gcc cct aaa ctc ctg atc tat aag gcc tct agt tta gcc    242
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser Leu Ala
      65          70          75

```

-continued

agt ggg gcc cca tca agg ttc agc ggc agt gga tct ggg aca gat ttc Ser Gly Ala Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe 80 85 90	290
act ctc acc atc agc agc ctg cag cct gat gat ttt gca act tat tac Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr 95 100 105	338
tgc caa caa tat agt aat tat ccg ctc act ttc ggc gga ggg acc aag Cys Gln Gln Tyr Ser Asn Tyr Pro Leu Thr Phe Gly Gly Thr Lys 110 115 120 125	386
ctg gag atc aaa cgtgagtgga tcctaga Leu Glu Ile Lys	415
<p><210> SEQ ID NO 76 <211> LENGTH: 24 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: DNA encoding a FLAG tag sequence <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(24)</p>	
<400> SEQUENCE: 76	
gac tac aag gat gac gac gat aag Asp Tyr Lys Asp Asp Asp Asp Lys 1 5	24
<p><210> SEQ ID NO 77 <211> LENGTH: 31 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>	
<400> SEQUENCE: 77	
atagaattcc accatggagt ttgggctgag c	31
<p><210> SEQ ID NO 78 <211> LENGTH: 24 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>	
<400> SEQUENCE: 78	
tgaagagacg gtgaccattg tccc	24
<p><210> SEQ ID NO 79 <211> LENGTH: 28 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>	
<400> SEQUENCE: 79	
ggacaatggt caccgtctct tcagggtg	28
<p><210> SEQ ID NO 80 <211> LENGTH: 32 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Primer</p>	

-continued

<400> SEQUENCE: 80
ggagactggg tcactctggat gtccgatccg cc 32

<210> SEQ ID NO 81
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 81
gacatccaga tgaccagtc tcc 23

<210> SEQ ID NO 82
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 82
attgcgccg cttatcactt atcgtcgtca tcctttagt ctttgatctc cagcttgg 59

<210> SEQ ID NO 83
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: DNA
encoding a linker amino acid sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(45)

<400> SEQUENCE: 83
ggt ggt ggt ggt tcg ggt ggt ggt ggt tcg ggt ggt ggc gga tcg 45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 84
<211> LENGTH: 823
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(809)
<223> OTHER INFORMATION: pCOS-sc12B5. sc12B5

<400> SEQUENCE: 84
aagcttccac c atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt 50
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu
1 5 10

tta aga ggt gtc cag tgt cag gtg cag ctg gtg cag tct ggg gga ggc 98
Leu Arg Gly Val Gln Cys Gln Val Gln Leu Val Gln Ser Gly Gly Gly
15 20 25

ttg gtc cgg ccc ggg ggg tcc ctg agt ctc tcc tgt gca gtc tct gga 146
Leu Val Arg Pro Gly Gly Ser Leu Ser Leu Ser Cys Ala Val Ser Gly
30 35 40 45

atc acc ctc agg acc tac ggc atg cac tgg gtc cgc cag gct cca ggc 194
Ile Thr Leu Arg Thr Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

aag ggg ctg gag tgg gtg gca ggt ata tcc ttt gac gga aga agt gaa 242

-continued

Lys	Gly	Leu	Glu	Trp	Val	Ala	Gly	Ile	Ser	Phe	Asp	Gly	Arg	Ser	Glu	
			65					70							75	
tac	tat	gca	gac	tcc	gtg	cag	ggc	cga	ttc	acc	atc	tcc	aga	gac	agt	290
Tyr	Tyr	Ala	Asp	Ser	Val	Gln	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Ser	
		80					85					90				
tcc	aag	aac	acc	ctg	tat	ctg	caa	atg	aac	agc	ctg	aga	gcc	gag	gac	338
Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	
	95					100					105					
acg	gct	gtg	tat	tac	tgt	gcg	aga	gga	gca	cat	tat	ggg	ttc	gat	atc	386
Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Ala	His	Tyr	Gly	Phe	Asp	Ile	
110					115					120					125	
tgg	ggc	caa	ggg	aca	atg	gtc	acc	gtc	tcg	agt	ggg	ggg	ggg	tcg		434
Trp	Gly	Gln	Gly	Thr	Met	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	
				130					135					140		
ggg	ggg	ggg	ggg	tcg	ggg	ggg	ggc	gga	tcg	gac	atc	cag	atg	acc	cag	482
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Met	Thr	Gln	
			145				150						155			
tct	cct	tcc	acc	ctg	tct	gca	tct	att	gga	gac	aga	gtc	acc	atc	acc	530
Ser	Pro	Ser	Thr	Leu	Ser	Ala	Ser	Ile	Gly	Asp	Arg	Val	Thr	Ile	Thr	
		160				165						170				
tgc	cgg	gcc	agc	gag	ggg	att	tat	cac	tgg	ttg	gcc	tgg	tat	cag	cag	578
Cys	Arg	Ala	Ser	Glu	Gly	Ile	Tyr	His	Trp	Leu	Ala	Trp	Tyr	Gln	Gln	
	175					180					185					
aag	cca	ggg	aaa	gcc	cct	aaa	ctc	ctg	atc	tat	aag	gcc	tct	agt	tta	626
Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Ala	Ser	Ser	Leu	
190					195					200					205	
gcc	agt	ggg	gcc	cca	tca	agg	ttc	agc	ggc	agt	gga	tct	ggg	aca	gat	674
Ala	Ser	Gly	Ala	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	
				210					215					220		
ttc	act	ctc	acc	atc	agc	agc	ctg	cag	cct	gat	gat	ttt	gca	act	tat	722
Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Asp	Asp	Phe	Ala	Thr	Tyr	
			225					230					235			
tac	tgc	caa	caa	tat	agt	aat	tat	ccg	ctc	act	ttc	ggc	gga	ggg	acc	770
Tyr	Cys	Gln	Gln	Tyr	Ser	Asn	Tyr	Pro	Leu	Thr	Phe	Gly	Gly	Gly	Thr	
		240					245					250				
aag	ctg	gag	atc	aaa	gac	tac	aag	gat	gac	gac	gat	aag	tgataagcgg			819
Lys	Leu	Glu	Ile	Lys	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys				
	255					260						265				
ccgc																823

<210> SEQ ID NO 85
 <211> LENGTH: 131
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 5
 <400> SEQUENCE: 85

Met	Lys	Leu	Pro	Val	Arg	Leu	Leu	Val	Leu	Met	Phe	Trp	Ile	Pro	Ala
1				5						10				15	
Ser	Ser	Ser	Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val
			20					25					30		
Ser	Leu	Gly	Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu
		35					40					45			
Leu	His	Ser	Lys	Gly	Asn	Thr	Tyr	Leu	Gln	Trp	Tyr	Leu	Gln	Lys	Pro
	50					55					60				
Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser

-continued

```

65              70              75              80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                85                90                95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
                100               105               110
Ser Gln Ser Thr His Val Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu
                115               120               125
Glu Ile Lys
                130

```

```

<210> SEQ ID NO 86
<211> LENGTH: 136
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 6

```

```

<400> SEQUENCE: 86

```

```

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

```

```

<210> SEQ ID NO 87
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 7

```

```

<400> SEQUENCE: 87

```

```

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

```

-continued

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp Phe Thr
 85 90 95

Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 100 105 110

Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
 115 120 125

Glu Ile Lys
 130

<210> SEQ ID NO 88
 <211> LENGTH: 136
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 8

<400> SEQUENCE: 88

Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60

Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn
 65 70 75 80

Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr
 85 90 95

Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
 100 105 110

Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln
 115 120 125

Gly Thr Thr Leu Thr Val Ser Ser
 130 135

<210> SEQ ID NO 89
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 223> OTHER INFORMATION: Description of Artificial Sequence: linker amino
 acid sequence encoded by SEQ ID NO: 19

<400> SEQUENCE: 89

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

<210> SEQ ID NO 90
 <211> LENGTH: 274
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 20

<400> SEQUENCE: 90

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
 1 5 10 15

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

-continued

```

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

```

<210> SEQ ID NO 91
<211> LENGTH: 271
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 23
<400> SEQUENCE: 91
    
```

```

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


-continued

```

Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Ser Pro Leu
145                               150                               155                               160
Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser
                               165                               170                               175
Ser Gln Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr
                               180                               185                               190
Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
                               195                               200                               205
Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val
210                               215                               220
Thr Asp Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly
225                               230                               235
Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly
                               245                               250                               255
Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp Asp Asp Lys
                               260                               265                               270

```

<210> SEQ ID NO 94

<211> LENGTH: 150

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<220> FEATURE:

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 26

<400> SEQUENCE: 94

```

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

```

<210> SEQ ID NO 95

<211> LENGTH: 245

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<220> FEATURE:

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 29

<400> SEQUENCE: 95

```

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

```

-continued

```

Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln
145                150                155                160
Ser Leu Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln
                165                170                175
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
                180                185                190
Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp
                195                200                205
Phe Thr Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr
                210                215                220
Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr
225                230                235                240
Lys Leu Glu Ile Lys
                245

```

<210> SEQ ID NO 96

<211> LENGTH: 533

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<220> FEATURE:

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 32

<400> SEQUENCE: 96

```

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

```

-continued

```

Met Thr Gln Ser Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala
      405                410                415
      420                425                430
Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Lys
      435                440                445
Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu
      450                455                460
Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
465                470                475                480
Ser Gly Ser Gly Ser Val Thr Asp Phe Thr Leu Met Ile Ser Arg Val
      485                490                495
Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val
      500                505                510
Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys
      515                520                525
Asp Asp Asp Asp Lys
      530

```

```

<210> SEQ ID NO 97
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 48

```

```

<400> SEQUENCE: 97

```

```

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

```

```

<210> SEQ ID NO 98
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 54

```

```

<400> SEQUENCE: 98

```

```

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Gly
 1                5                10                15
Ser Ser Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
      20                25                30
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu

```

-continued

```

      35          40          45
Val His Ser Asn Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
  50          55          60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
  65          70          75          80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp Phe Thr
      85          90          95
Leu Met Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
      100          105          110
Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
      115          120          125
Glu Ile Lys Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
      130          135          140
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
      145          150          155          160
Ala Asn His Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
      165          170          175
Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn
      180          185          190
Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Thr
      195          200          205
Thr Ala Tyr Met Asp Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
      210          215          220
Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln
      225          230          235          240
Gly Thr Thr Leu Thr Val Ser Ser Asp Tyr Lys Asp Asp Asp Asp Lys
      245          250          255

```

```

<210> SEQ ID NO 99
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 55

```

<400> SEQUENCE: 99

```

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

```

```

<210> SEQ ID NO 100
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Leader sequence encoded by SEQ ID NO: 56

```

<400> SEQUENCE: 100

```

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
  1          5          10          15
Val Gln Cys

```

```

<210> SEQ ID NO 101
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:

```

-continued

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 63

<400> SEQUENCE: 101

```

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

```

<210> SEQ ID NO 102

<211> LENGTH: 136

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 66

<400> SEQUENCE: 102

```

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

```

<210> SEQ ID NO 103

<211> LENGTH: 107

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 67

<400> SEQUENCE: 103

```

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

```

<210> SEQ ID NO 104

<211> LENGTH: 22

-continued

<212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Leader sequence encoded by SEQ ID NO: 68

<400> SEQUENCE: 104

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

Leu Pro Gly Ala Lys Cys
 20

<210> SEQ ID NO 105
 <211> LENGTH: 129
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 75

<400> SEQUENCE: 105

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

Leu Pro Gly Ala Lys Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Thr
 20 25 30

Leu Ser Ala Ser Ile Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
 35 40 45

Glu Gly Ile Tyr His Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
 50 55 60

Ala Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser Leu Ala Ser Gly Ala
 65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 85 90 95

Ile Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
 100 105 110

Tyr Ser Asn Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 115 120 125

Lys

<210> SEQ ID NO 106
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: FLAG tag
 sequence encoded by SEQ ID NO: 76

<400> SEQUENCE: 106

Asp Tyr Lys Asp Asp Asp Asp Lys
 1 5

<210> SEQ ID NO 107
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: linker
 amino acid sequence encoded by SEQ ID NO: 83

<400> SEQUENCE: 107

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

<210> SEQ ID NO 108
 <211> LENGTH: 266
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: amino acid sequence encoded by SEQ ID NO: 84

-continued

<400> SEQUENCE: 108

```

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

<210> SEQ ID NO 109

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(27)

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic DNA

<400> SEQUENCE: 109

```

gtc tcg agt ggt ggt tcc gac gtc gtg
Val Ser Ser Gly Gly Ser Asp Val Val
 1          5
    
```

27

<210> SEQ ID NO 110

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 110

```

Val Ser Ser Gly Gly Ser Asp Val Val
 1          5
    
```

<210> SEQ ID NO 111

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

```

<221> NAME/KEY: CDS
<222> LOCATION: (1)..(30)
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      DNA

<400> SEQUENCE: 111

gtc tcg agt ggt ggt ggt tcc gac gtc gtg           30
Val Ser Ser Gly Gly Ser Asp Val Val
  1             5             10

<210> SEQ ID NO 112
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 112

Val Ser Ser Gly Gly Gly Ser Asp Val Val
  1             5             10

<210> SEQ ID NO 113
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(33)
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      DNA

<400> SEQUENCE: 113

gtc tcg agt ggt ggt ggt ggt tcc gac gtc gtg           33
Val Ser Ser Gly Gly Gly Gly Ser Asp Val Val
  1             5             10

<210> SEQ ID NO 114
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 114

Val Ser Ser Gly Gly Gly Gly Ser Asp Val Val
  1             5             10

<210> SEQ ID NO 115
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(36)
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      DNA

<400> SEQUENCE: 115

gtc tcg agt ggt ggt ggt ggt tcc gac gtc gtg           36
Val Ser Ser Gly Gly Gly Gly Ser Asp Val Val
  1             5             10

```

-continued

<210> SEQ ID NO 116
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

 <400> SEQUENCE: 116

 Val Ser Ser Gly Gly Gly Gly Ser Asp Val Val
 1 5 10

<210> SEQ ID NO 117
 <211> LENGTH: 39
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(39)
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic DNA

 <400> SEQUENCE: 117

 gtc tcg agt ggt ggt ggt ggt ggt tcc gac gtc gtc
 Val Ser Ser Gly Gly Gly Gly Gly Ser Asp Val Val
 1 5 10 39

<210> SEQ ID NO 118
 <211> LENGTH: 13
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

 <400> SEQUENCE: 118

 Val Ser Ser Gly Gly Gly Gly Gly Gly Ser Asp Val Val
 1 5 10

<210> SEQ ID NO 119
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(27)
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic DNA

 <400> SEQUENCE: 119

 gag ata aaa tcc gga ggc cag gtc caa
 Glu Ile Lys Ser Gly Gly Gln Val Gln
 1 5 27

<210> SEQ ID NO 120
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

 <400> SEQUENCE: 120

 Glu Ile Lys Ser Gly Gly Gln Val Gln

-continued

1 5

<210> SEQ ID NO 121
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(30)
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 DNA

<400> SEQUENCE: 121

gag ata aaa tcc gga ggt ggc cag gtc caa 30
 Glu Ile Lys Ser Gly Gly Gln Val Gln
 1 5 10

<210> SEQ ID NO 122
 <211> LENGTH: 10
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 peptide

<400> SEQUENCE: 122

Glu Ile Lys Ser Gly Gly Gln Val Gln
 1 5 10

<210> SEQ ID NO 123
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(33)
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 DNA

<400> SEQUENCE: 123

gag ata aaa tcc gga ggt ggt ggc cag gtc caa 33
 Glu Ile Lys Ser Gly Gly Gly Gln Val Gln
 1 5 10

<210> SEQ ID NO 124
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 peptide

<400> SEQUENCE: 124

Glu Ile Lys Ser Gly Gly Gly Gln Val Gln
 1 5 10

<210> SEQ ID NO 125
 <211> LENGTH: 36
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(36)
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

DNA

<400> SEQUENCE: 125

gag ata aaa tcc gga ggt ggt ggt ggc cag gtc caa 36
Glu Ile Lys Ser Gly Gly Gly Gly Gly Gln Val Gln
1 5 10

<210> SEQ ID NO 126

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 126

Glu Ile Lys Ser Gly Gly Gly Gly Gly Gln Val Gln
1 5 10

<210> SEQ ID NO 127

<211> LENGTH: 39

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(39)

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic DNA

<400> SEQUENCE: 127

gag ata aaa tcc gga ggt ggt ggt ggt ggc cag gtc caa 39
Glu Ile Lys Ser Gly Gly Gly Gly Gly Gly Gln Val Gln
1 5 10

<210> SEQ ID NO 128

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 128

Glu Ile Lys Ser Gly Gly Gly Gly Gly Gln Val Gln
1 5 10

<210> SEQ ID NO 129

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 129

Gly Gly Gly Ser
1

<210> SEQ ID NO 130

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

-continued

<400> SEQUENCE: 130

Ser Gly Gly Gly

1

<210> SEQ ID NO 131

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 131

Gly Gly Gly Gly Ser

1

5

<210> SEQ ID NO 132

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 132

Ser Gly Gly Gly Gly

1

5

<210> SEQ ID NO 133

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 133

Gly Gly Gly Gly Gly Ser

1

5

<210> SEQ ID NO 134

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 134

Ser Gly Gly Gly Gly Gly

1

5

<210> SEQ ID NO 135

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 135

Gly Gly Gly Gly Gly Gly Ser

1

5

-continued

<210> SEQ ID NO 136
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 136

Ser Gly Gly Gly Gly Gly Gly
 1 5

<210> SEQ ID NO 137
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: PEPTIDE
 <222> LOCATION: (1)..(40)
 <223> OTHER INFORMATION: This peptide may range from 1-40 amino acids; refer to the specification as filed for preferred embodiments

<400> SEQUENCE: 137

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser
 35 40

<210> SEQ ID NO 138
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: PEPTIDE
 <222> LOCATION: (1)..(40)
 <223> OTHER INFORMATION: This peptide may range from 1-40 amino acids; refer to the specification as filed for preferred embodiments

<400> SEQUENCE: 138

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 20 25 30

Gly Gly Gly Ser Gly Gly Gly Gly
 35 40

What is claimed is:

1. A modified antibody comprising two or more H chain V regions and two or more L chain V regions of monoclonal antibody and showing an agonist action by crosslinking a cell surface molecule(s).

2. The modified antibody of claim 1, wherein H chain V region and L chain V region are connected through a linker.

3. The modified antibody of claim 1 or 2, wherein the linker comprises at least one amino acid.

4. The modified antibody of any one of claims 1 to 3, wherein the modified monoclonal antibody is a dimer of single chain Fv comprising an H chain V region and an L chain V region.

5. The modified antibody of any one of claims 1 to 3, wherein the modified antibody is a single chain polypeptide comprising two H chain V regions and two L chain V regions.

6. The modified antibody of any one of claims 1 to 5, wherein the modified antibody further comprises an amino acid sequence(s) for peptide purification.

7. The modified antibody of any one of claims 1 to 6, wherein the modified antibody has been purified.

8. The modified antibody of any one of claims 1 to 7, wherein H chain V region and/or L chain V region is humanized H chain V region and/or L chain V region.

9. The modified antibody of any one of claims 1 to 8, wherein the cell surface molecule is a hormone receptor or a cytokine receptor.

10. The modified antibody of claim 9, wherein the cell surface molecule is selected from the group consisting of erythropoietin (EPO) receptor, thrombopoietin (TPO) receptor, granulocyte colony stimulating factor (G-CSF) receptor, macrophage colony stimulating factor (M-CSF) receptor, granular macrophage colony stimulating factor (GM-CSF) receptor, tumor necrosis factor (TNF) receptor, interleukin-1 (IL-1) receptor, interleukin-2 (IL-2) receptor, interleukin-3 (IL-3) receptor, interleukin-4 (IL-4) receptor, interleukin-5 (IL-5) receptor, interleukin-6 (IL-6) receptor, interleukin-7 (IL-7) receptor, interleukin-9 (IL-9) receptor, interleukin-10 (IL-10) receptor, interleukin-11 (IL-11) receptor, interleukin-12 (IL-12) receptor, interleukin-13 (IL-13) receptor, interleukin-15 (IL-15) receptor, interferon-alpha (IFN-alpha) receptor, interferon-beta (IFN-beta) receptor, interferon-gamma (IFN-gamma) receptor, growth hormone (GH) receptor, insulin receptor, blood stem cell proliferation factor (SCF) receptor, vascular epidermal growth factor (VEGF) receptor, epidermal cell growth factor (EGF) recep-

tor, nerve growth factor (NGF) receptor, fibroblast growth factor (FGF) receptor, platelet-derived growth factor (PDGF) receptor, transforming growth factor-beta (TGF-beta) receptor, leukocyte migration inhibitory factor (LIF) receptor, ciliary neurotrophic factor (CNTF) receptor, oncostatin M (OSM) receptor and Notch family receptor.

11. The modified antibody of any one of claims 1 to 10, wherein the agonist action is induction of apoptosis, induction of cell proliferation and induction of cell differentiation.

12. The monoclonal antibody of any one of claims 1 to 11, wherein the L chain V region and the H chain V region are from the same monoclonal antibody.

13. The monoclonal antibody of any one of claims 1 to 12 which shows an improved agonist action compared with the original monoclonal antibody.

14. A DNA which encodes the modified antibody of any one of claims 1 to 13.

15. An animal cell which produces the modified antibody of any one of claims 1 to 13.

16. A microorganism which produces the modified antibody of any one of claims 1 to 13.

17. Use of the modified antibody of any one of claims 1 to 13 as an agonist.

18. A method of producing a dimer of single chain Fv which comprises culturing host animal cells producing the single chain Fv in serum-free medium to have the single chain Fv secreted into the medium and purifying a dimer of the single chain Fv produced in the medium.

19. A method of stabilizing a dimer of single chain Fv which comprises culturing host animal cells producing single chain Fv in serum-free medium to have the single chain Fv secreted into the medium and to form a dimer of the single chain Fv.

20. A method of inducing agonist action to cells which comprises administering the first ligand and the second ligand binding to a cell surface molecule(s) and administering a substance which binds to the first and the second ligands and crosslinks the first and the second ligands.

21. The method of claim 20 wherein the first and the second ligands are the same or different single chain Fv monomer.

22. The method of claim 20 or 21 wherein the substance which crosslinks the ligands is an antibody, an antibody fragment or a bivalent modified antibody.

* * * * *

专利名称(译)	激动剂抗体		
公开(公告)号	US20040258684A1	公开(公告)日	2004-12-23
申请号	US10/645085	申请日	2002-10-07
申请(专利权)人(译)	中外SEIYAKU株式会社		
当前申请(专利权)人(译)	中外SEIYAKU株式会社		
[标]发明人	FUKUSHIMA NAOSHI TSUCHIYA MASAYUKI OH EDA MASAYOSHI UNO SHINSUKE KIKUCHI YASUFUMI OHTOMO TOSHIHIKO		
发明人	FUKUSHIMA, NAOSHI TSUCHIYA, MASAYUKI OH-EDA, MASAYOSHI UNO, SHINSUKE KIKUCHI, YASUFUMI OHTOMO, TOSHIHIKO		
IPC分类号	A61K38/00 C07K16/28 C07K16/30 G01N33/53 A61K39/395 C07K16/44		
CPC分类号	A61K38/00 A61K2039/505 C07K16/28 C07K16/2866 C07K16/3061 C07K2317/21 C07K2317/24 C07K2317/31 C07K2317/56 C07K2317/622 C07K2317/73 C07K2319/00		
优先权	2000321822 2000-10-20 JP 2000321821 2000-10-20 JP 2000115246 2000-04-17 JP PCT/JP2001/001912 2001-03-12 WO		
外部链接	Espacenet USPTO		

摘要(译)

含有2个或更多H链V结构域和/或更多L链V结构域的单克隆抗体的修饰抗体，其可通过交联细胞表面分子将信号转导入细胞，从而用作激动剂。由于可用作信号转导的激动剂，这些修饰的抗体可用作例如癌症，炎症，激素紊乱和血液病等各种疾病的预防和/或治疗。

