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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 October 2012 (26.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/144692 A1

(51) International Patent Classification:
C07K 16/18 (2006.01) *C12N 15/13* (2006.01)
A61K 39/395 (2006.01) *G01N 33/53* (2006.01)

(21) International Application Number:
PCT/KR2011/005203

(22) International Filing Date:
14 July 2011 (14.07.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10-2011-0036569 20 April 2011 (20.04.2011) KR

(71) Applicant *(for all designated States except US)*: **CELL & BIO CO., LTD.** [KR/KR]; 2nd Floor, Daelim Store, 960, Dogok-dong, Gangnam-gu, Seoul 135-270 (KR).

(72) Inventor; and

(71) Applicant : **YOON, Kang-Jun** [KR/KR]; 101-603, Poscot APT., 164-6(28/8), Dogok-dong, Gangnam-gu, Seoul 135-270 (KR).

(74) Agent: **AHN, So-Young**; Dr. Ahn International Patent Law Office, 10th Fl., shinseung Bldg., 1676-1, Seocho-dong, Seocho-gu, Seoul 137-881 (KR).

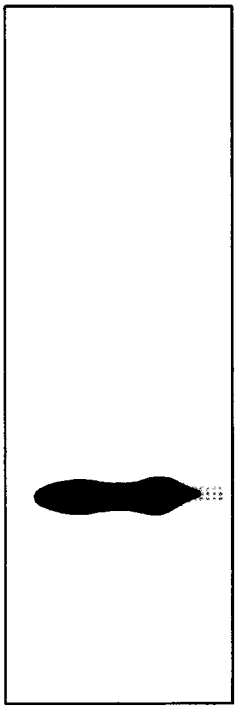
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG,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

[Continued on next page]

WO 2012/144692 A1

(54) Title: HUMANIZED ANTI-EMAP II ANTIBODY AND USE THEREOF

【Figure 1】



(57) Abstract: The present invention provides a humanized anti-EMAP II antibody, the use of the humanized antibody, and pharmaceutical compositions containing the humanized antibody. The humanized anti-EMAP II antibody shows reduced immunogenicity and increased half-life while having similar or improved antigen binding capacity compared to the parent monoclonal antibody. Thus, the humanized anti-EMAP II antibody of the present invention can be more effectively used as a diagnostic reagent for EMAP II and a therapeutic agent for diseases that are mediated by EMAP II.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

【DESCRIPTION】

【Invention Title】

HUMANIZED ANTI-EMAP II ANTIBODY AND USE THEREOF

【Technical Field】

<1> The present invention relates to a humanized anti-EMAP II antibody and the use thereof.

【Background Art】

<2> Endothelial monocyte activating polypeptide II (EMAP II) is a polypeptide isolated from methylcholanthrene A-transformed fibrosarcoma cells (Kao et al., J. Biol. Chem. 267:20239-20247, 1992). It is known to inhibit the growth of primary and metastatic tumors and induce apoptosis in proliferating endothelial cells (Schwarz et al., J. Exp. Med. 190:341-353, 1999).

<3> EMAP II is released from the precursor protein p43 during apoptosis. The precursor p43 (pro-EMAP II) protein consists of 312 amino acids and is associated with the multi-tRNA synthetase complex in eukaryotes (Park et al., J. Biol. Chem. 274:16673-166776, 1999). However, it is cleaved to release its C-terminal domain by an activated caspase-7 during apoptosis and to generate EMAP-II (Behrensdorf et al., FEBS Lett. 466:143-147, 2000).

<4> It is known that the EMAP II protein shows sequence similarity with domains present in a variety of different aminoacyl-tRNA synthetases (Quevillon et al., J. Biol. Chem. 272; 32573-32579, 1997) and can bind with tRNA. Also, it is known that 15 peptides at the N-terminal end are involved in the cytokine activity of EMAP II (Kao et al. J. Biol. Chem. 269:9774-9782, 1994).

<5> EMAP II is a mediator of proinflammatory responses that induces the expression of tissue factor, tumor necrosis factor (hereinafter referred to as "TNF") and interleukin-8 (hereinafter referred to as "IL-8") in mononuclear phagocyte and polymorphonuclear leucocytes. Also, in a tissue expressing a high level of EMAP II mRNA, macrophages are accumulated. This means that EMAP II is a chemotaxis material directing macrophage to dead cells. It is known that EMAP II acts as a cytokine, and 15 amino acids at

the N-terminal domain of EMAP II play a significant role (Quevillon, S. et al., J. Biol. Chem., 272:32573-32579, 1997; Kao, J. et al., J. Biol. Chem., 269:9774-7982, 1994; Kao, J. et al., J. Biol. Chem., 267:20239-20247, 1992; Kao, J. et al., J. Biol. Chem., 269:25106-25119, 1994; Knies, U. E. et al., PNAS USA, 95:12322-12327, 1998). In US Patent No. 5,641,867 discloses that the N-terminal domain of an EMAP II comprising arginine-isoleucine-glycine-arginine-isoleucine-threonine is an important residue in the cytokine function of the EMAP II. Recently, it was reported that EMAP II repressed the growth of primary and metastatic tumors in proliferating endothelial cells while it did not cause particular side effects in normal cells (Schwarz, M. A. et al., J. Exp. Med., 190:341-353, 1999).

<6> Meanwhile, p43 known as the precursor of EMAP II is known to be expressed extensively. The expression level of p43 protein varies temporally and spatially, especially in a developing mouse. For example, it was shown that the expression of p43 in the lung of 8-16-day-old mice was increased dramatically. In addition, p43 is highly expressed in the microglial cells in the lesions of autoimmune disease such as encephalomyelitis, neuritis and uveitis. The high expression level of p43 in specific developmental stages and tissues suggests that p43 involves inflammation and apoptosis (Tas, M. P. R., and Marray, J. C., Int. J. Biochem. Cell. Biol., 28:837-841, 1996; Schwarz, M. J. et al., Glia, 20:365-372, 1997; Schuesner, H. J. et al., Glia, 20:365-372, 1997; Berger, A. C. et al., J. Immunother., 23:519-527, 2000).

<7> Previously, the present inventor found that monoclonal antibodies specific for EMAP II can be used for the diagnosis and treatment of inflammatory diseases, inhibits the secretion of TNF-α mediating inflammatory responses and is effective for the treatment of Alzheimer's disease (Korean Patent Laid-Open Publication No. 10-2010-0093451).

【Disclosure】

【Technical Problem】

<8> Monoclonal antibodies (mAbs) have enormous potential as therapeutic agents. However, non-human antibodies are highly immunogenic in the human body and their short half-life severely limits their clinical efficacy.

【Technical Solution】

<9> Accordingly, the present inventor has made many efforts to solve the above-described problem of the humanized antibody and, as a result, has prepared an EMAP II-specific humanized antibody which has minimized immunogenicity while having similar or improved antigen-binding capacity compared to the parent monoclonal antibody that binds specifically to EMAP II, thereby completing the present invention.

<10> One aspect of the present invention provides a humanized anti-EMAP II antibody that binds specifically to EMAP II.

<11> Another aspect of the present invention provides the use of the humanized anti-EMAP II antibody.

<12> Still another aspect of the present invention provides a pharmaceutical composition including the humanized anti-EMAP II antibody.

<13> Mouse-derived antibodies induce undesired immune responses in humans, because they act as antigens in humans so that new human anti-mouse antibodies (HAMAs) against the mouse-derived antibodies are produced. To overcome this problem, proposals have been made for reducing the immunogenicity of non-human antibodies in humans. Such techniques can be generically termed "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule. Early methods for preparing humanized antibodies involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of a non-human antibody is linked to constant domains derived from a human antibody.

<14> Accordingly, the present invention provides a chimeric anti-EMAPII antibody comprising: a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 11; and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 19.

<15> The present invention also provides a humanized form of the chimeric anti-EMAPII antibody.

<16> Specifically, the present invention provides a humanized anti-EMAPII

(endothelial monocyte activating polypeptide II) comprising: (i) a light-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 12 to SEQ ID NO: 18; and (ii) a heavy-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 20 to SEQ ID NO: 26.

<17> In one embodiment, the present invention provides a humanized anti-EMAPII antibody comprising: a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 12, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 20; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 13, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 21; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 14, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 22; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 15, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 23; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 16, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 24; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 17, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 25; or a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 18, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 26.

<18> As used herein, the term "humanized antibody" generally means an antibody that is non-immunogenic in humans or has reduced immunogenicity in humans. A humanized antibody is an antibody having an altered amino acid sequence, and the amino acid sequence of the antibody may be reconstituted according to intended purposes. A large number of changes are possible, which range from changes of one or several amino acids to complete reconstitution of variable and/or constant regions of an antibody.

<19> As used herein, the term "variable domain" means a portion of an

antibody molecule, which functions to bind specifically to an antigen and shows many variations in its sequence. Complementarity determining regions, CDR1, CDR2 and CDR3, are present in a variable domain. The term "complementarity determining regions (CDRs)" means loop-like regions which participate in antigen recognition and in which the specificity of an antibody for an antigen is determined according to changes in the sequences of CDRs.

<20> The present invention also provides a humanized anti- EMAPII antibody comprising, in addition to said light-chain variable domain and heavy-chain variable domain, a human IgG1-derived constant domain.

<21> The present invention also provides a pharmaceutical composition comprising said humanized anti-EMAPII antibody.

<22> The present invention also provides a kit for quantification of EMAPII comprising said humanized anti-EMAPII antibody.

<23> The present invention also provides a nucleic acid molecule encoding a light-chain variable domain having an amino acid sequence set forth in any one SEQ ID NO: 12 to SEQ ID NO: 18.

<24> The present invention also provides a nucleic acid molecule encoding a heavy-chain variable domain having an amino acid sequence set forth in any one SEQ ID NO: 20 to SEQ ID NO: 26.

<25> The present invention also provides a recombinant vector comprising nucleic acid molecules encoding said light-chain variable domain and heavy-chain variable domain.

<26> The present invention also provides a transformed cell comprising said recombinant vector.

<27> The present invention also provides a method of producing said humanized antibody by culturing said transformed cell.

<28> "Humanized forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In most instances, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-

human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Reichmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol . 2:593-596 (1992).

<29> Methods for humanizing non-human antibodies are well known in the art, and a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. Typically, humanized antibodies are human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

<30> The choice of human variable domains, both light and heavy chains, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire

library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993)).

<31> It is also important to humanize antibodies with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the residues in the hypervariable region are directly and most substantially involved in influencing antigen binding.

<32> Modifications of the antibody are also contemplated in the present invention. For example, the antibody can be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol.

<33> The antibody also can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

<34> The anti-EMAP II antibody of the present invention can be formulated as immunoliposomes. Liposomes containing the antibody can be prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA 77:4030 (1980); US Patent Nos. 4,485,045 and 4,544,545; and WO97/38731 published Oct. 23, 1997. Liposomes with enhanced circulation time are disclosed in US Patent No. 5,013,556.

<35> Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem. 257: 286-88 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome (Gabizon et al. J. National Cancer Inst. 81 (19) 1484 (1989)).

<36> The present invention also provides an isolated nucleic acid encoding the humanized anti-EMAP II antibody, a recombinant vector containing the nucleic acid, a transformed cell comprising the recombinant vector and a recombinant technique for producing the antibody.

<37> For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures

(e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Conventional vectors known in the art are available. The vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

<38> The present invention also provides a microbial or animal cell transformed with said recombinant vector. A microbial or animal cell that can be transformed with the vector in the present invention may be a known microbial or animal cell for transformation which is used in the art.

<39> The present invention also provides pharmaceutical formulations comprising the humanized anti-EMAP II antibody. The pharmaceutical formulations of the present invention are prepared for storage by mixing the antibody that has the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington' s Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or

polyethylene glycol (PEG).

<40> The humanized anti-EMAPII antibody of the present invention effectively inhibits the secretion of TNF-α, and thus can be used for prevention or treatment of TNF-α-mediated diseases.

<41> TNF-α-mediated diseases include adult respiratory distress syndrome; anorexia; cancer (e.g., leukemia); chronic fatigue syndrome; graft versus host rejection; hyperalgesia; inflammatory bowel disease; neuroinflammatory diseases; ischemic/reperfusion injury, including cerebral ischemia (brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration); diabetes (e.g., juvenile onset Type 1 diabetes mellitus); multiple sclerosis; ocular diseases; pain; pancreatitis; pulmonary fibrosis; rheumatic diseases (e.g., rheumatoid arthritis, osteoarthritis, juvenile (rheumatoid) arthritis, seronegative polyarthritis, ankylosing spondylitis, Reiter's syndrome and reactive arthritis, psoriatic arthritis, enteropathic arthritis, polymyositis, dermatomyositis, scleroderma, systemic sclerosis, vasculitis, cerebral vasculitis, Sjogren's syndrome, rheumatic fever, polychondritis and polymyalgia rheumatica and giant cell arteritis); septic shock; side effects from radiation therapy; systemic lupus erythematous; temporal mandibular joint disease; and thyroiditis.

<42> In addition, the humanized anti-EMAPII antibody of the present invention can be used for prevention or treatment of Alzheimer's disease.

<43> The anti-EMAPII antibody of the present invention can be administered to a human patient by a known method in a bolus dose or by continuous injection over a certain period of time, for example, via an intravenous, intramuscular, intraperitoneal, intracerebral, subcutaneous, intraarticular, intrasynovial, intrasubarachnoidal, oral, local, or inhalation route. In a preferred embodiment, the antibody is administered intravenously or subcutaneously.

<44> For the prevention or treatment of disease, the appropriate dosage of the antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, previous therapy, the

patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 10μg/kg to 5mg/kg of the antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. The typical daily dose of the humanized anti-EMAPII antibody of the present invention may range from 10 μg/kg to 5 mg/kg depending on the above-mentioned factors. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms is achieved. The preferred dosage of the antibody will be in the range from about 1 mg/kg to about 2 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 1 mg/kg or 2 mg/kg (or any combination thereof) may be administered to the patient.

<45> Aside from administration of the antibody protein to the patient, the present invention contemplates administration of the antibody by gene therapy. See, for example, WO 96/07321 published Mar. 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

<46> The present invention also provides an article containing the humanized anti-EMAPII antibody. This article comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials or syringes. The composition of the present invention may be placed into a container with a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

<47> The humanized anti-EMAPII antibody of the present invention may also be used for non-therapeutic purposes.

<48> For example, the antibody of the present invention may be used as affinity purification agents. In this process, the antibody is immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing EMAP II protein (or a fragment thereof) to be purified, and then

the support is washed with a suitable solvent that will remove substantially all the material in the sample except the EMAP II protein, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer (pH 5.0) that will release the EMAP II protein from the antibody.

<49> Also, the anti-EMAPII antibody may be useful for a diagnostic assay for EMAPII protein, for example, for detecting the expression of EMAPII protein in a specific cell, tissue or serum.

<50> When the antibody is used for diagnosis, it will be labeled with detectable labels. Such labels include: (a) radioactive isotopes such as ^{35}S, ^{14}C, ^{125}I, ^{3}H and ^{131}I (see, for example, Current Protocols in Immunology, Volumes 1 and 2, Coligen et al., Ed. Wiley-Interscience, New York, New York, Pubs. (1991)); (b) fluorescent labels, including rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red (see, for example, Current Protocols in Immunology, supra); (c) enzymatic labels, including luciferases (e.g., firefly luciferase and bacterial luciferase; US Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase (e.g., horseradish peroxidase (HRPO)), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocydic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like (see, for example, US Patent No. 4,275,149, O'Sullivan et a., Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym. (ed J. Langone & H.Van Vunakis), Academic press, New York, 73:147-166 (1981)).

<51> The detectable label can be indirectly conjugated with the antibody. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin, and thus the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is

conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

<52> Additionally, the antibody of the present invention needs not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the antibody of the present invention.

<53> The antibody of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Zola, Monoclonal Antibodies: A Manual of Techniques, at pp.147-158 (CRC Press, Inc. 1987)).

<54> For immunohistochemistry, a tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin.

<55> The antibody of the present invention may also be used for in vivo diagnostic assays. Generally, the antibody is labeled with a radio nuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, ^{32}P or ^{35}S) so that tumors can be localized using immunoscintiography.

<56> As a matter of convenience, the antibody of the present invention can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts in one or more containers with instructions for performing the diagnostic assay. Where the antibody is labeled with an enzyme, the kit will include substrates and co factors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

【Advantageous Effects】

<57> The humanized anti-EMAP II antibody of the present invention, which

binds specifically to EMAP II, shows reduced immunogenicity and increased half-life while having similar or improved antigen binding capacity compared to the parent monoclonal antibody. Thus, the humanized anti-EMAP II antibody of the present invention can be more effectively used as a diagnostic reagent for EMAP II and a therapeutic agent for diseases that are mediated by EMAP II.

【Description of Drawings】

<58> FIG. 1 shows the results of confirming that a monoclonal antibody cell line of the present invention is specific for EMAPII.

<59> FIG. 2 shows the results of determining the isotype of the monoclonal antibody of the present invention.

<60> FIG. 3 shows the results of PCR carried out using a combination of kappa-specific primers for the light-chain variable domain of mouse anti-EMAPII monoclonal antibody.

<61> FIG. 4 shows the results of PCR carried out using a combination of heavy chain-specific primers for the heavy-chain variable domain of mouse anti-EMAPII monoclonal antibody.

<62> FIG. 5 shows the results of sequencing a PCR product obtained using a primer combination of mK2 and mK3 (top: Genbank entry AAA39004).

<63> FIG. 6 shows the results of sequencing clones induced using the primers mk6, mK7, mK8, mK9 and mK11 (aligned with the mouse embryonic gene Vk 19-17).

<64> FIG. 7 shows the results of performing variable-domain PCR using gene-specific primers.

<65> FIG. 8 shows the DNA and deduced amino acid sequences of a light-chain variable domain, obtained by PCR.

<66> FIG. 9 shows the results of sequencing a variable-domain PCR product obtained using the primer H4.

<67> FIG. 10 shows the results of sequencing clones induced using a primer combination of H1, H2, H3, H5, H6 and H7 (aligned with the mouse embryonic gene Vh1-4*1).

<68> FIG. 11 shows the DNA and deduced amino acid sequences of a heavy-chain variable domain, obtained by PCR.

<69> FIG. 12 is a graph showing the results of determining the optimized ELISA conditions of mouse anti-EMAPII antibody.

<70> FIG. 13 is a graph showing the binding affinity of chimeric anti-EMAPII antibody in comparison with that of mouse anti-EMAPII monoclonal antibody.

<71> FIG. 14 is a graph showing the specific activities of recombinant humanized clones to antigen.

<72> FIG. 15 is a graph showing the specific activities of the selected 1st HITs.

<73> FIG. 16 is a graph showing the specific activities of top HITs to antigen.

<74> FIG. 17 shows the amino acid sequences of humanized anti-EMAPII antibodies of seven top HITs among recombinant humanized clones.

【Mode for Invention】

<75> Hereinafter, the present invention will be described in detail with reference to examples. It is to be understood, however, that these examples are for illustrative purposes only and are not to be construed to limit the scope of the present invention.

<76> [Examples]

<77> **Example 1. Manufacturing of monoclonal anti-EMAPII antibody-producing hybridoma**

<78> **(1) Cloning EMAPII and separation of protein**

<79> ORF(1-312aa) encoding human EMAPII (SEQ ID No. 1) was amplified by using primer; F: 5'-GCGAATTCATGGCAAATAATGATGCTGTTC-3' (SEQ ID No. 2), R: 5'-CCGCTCGAGTTATTTGATTCCACTGTTGCTCATG-3' (SEQ ID NO. 3) from HEK293 cDNA and performing 25 cycles of 95℃ 1 min, 55℃ 1 min and 72℃ 1 min. The PCR product was restricted by EcoRI/SalI, and ligased with pGEX4T-1 vector (Pharmacia), formerly restricted with EcoRI/SalI, for 2 hours at room temperature. After transformation of DH-5a by heat shock for 90 sec at 42℃, the E. coli was cultured in LB media containing ampiciline (50㎍/ml), and colonies including EMAPII were selected. The cloned EMAPII gene was sequenced and there was no mutation.

<80> After transformation of E. coli BL21 (DE3) with EMAPII plasmid, the E.

coli was plated to LB media containing ampiciline (50㎍/ml), and cultured for 24 hours at 37℃. The grown colony was cultured in 5 ml of LB media containing ampiciline (50 ㎍/ml) for 12 hours, and then 4ml of the cultured media was inoculated to 2L of LB media containing ampiciline (50㎍/ml). It was cultured at 37℃ until O.D (600nm) reached 0.3. IPTG was added in a final concentration of 0.1mM, and p43 protein was expressed by culturing for 6 hours at 30℃. After E. coli was centrifuged for 15 min at 7,000 rpm, the cells were resuspended by 1XPBS (8g NaCl, 0.2g KCl, 1.44g Na_2HPO, 0.24g KH_2PO_4/L, pH 7.4), sonicated at 4℃ and centrifuged at 4℃ for 40 min at 26,000 x g. The supernatant was obtained and loaded to a glutathione Sepharose 4B-column which becomes homogeneous by 1xPBS. The resin was washed with PBS, and GST-p43 was eluted by using 50mM of Tris-HCl (pH 8.0) containing 10mM of glutathione. The purity was identified by using 10% SDS-PAGE. The purity was over 90% on SDS-PAGE. The dialysis was performed by using PBS containing 20% glycerol, and the dialyzed product was stored at -70 ℃.

<81> (2) Preparation of the EMAPII antibody monoclone cell line

<82> 1) Antigen immunization

<83> An emulsion was prepared by mixing 20㎍/mouse of the protein with the same volume of Complete Freund's Adjuvant (Sigma, USA). The emulsion was intraperitoneally injected into three 7-week-old female Balb/c mice (orient). 20㎍ of antigen was injected into each mouse with the 400㎕ of total volumn. After 2 weeks, the emulsion mixing Incomplete Freund's Adjuvant (Sigma, USA) and antigen was intraperitoneally injected into the mouse. After 2 weeks, the antigen (20㎍/mouse) dissolved in PBS was intraperitoneally injected to induce the production of antibody. After identifying the antibody by performing enzyme immunoassay and western blot analysis, antigen dissolved in PBS was further injected into a tail vein of the mouse 3 days before the cell fusion.

<84>

<85> 2) Identifying and screening the cells producing antibody

<86> Blood was obtained from the eye ball of an immunized mouse according to

the above method, placed into the 1.5mℓ microcentrifuge tube and centrifuged for 10 min at 13,000 rpm. The serum was separated and stored at -20℃ until the experiment for identifying the production of antibody was performed. After identifying the production of antibody by performing enzyme immunoassay and western blot analysis using antigen protein, the fusion for spleen cells of the antibody-producing mouse was performed.

<87>

<88> 3) Preparation of hybridoma cell

<89> After the production of the antibody was identified, the mouse was sacrificed and the splenocytes were separated. The splenocytes were fused with myeloma cells P3X63Ag8.653 (ATCC CRL-1580, USA). That is, P3X63Ag8.653 cells of the mouse were maintained in the culture plate by using RPMI1640 media supplemented with 10% fetal bovine serum. To perform the cell fusion, P3X63Ag 8.653 cells were washed two times with serum-free RPMI1640 media (Biowhittaker, USA) and adjusted to become the concentration of 1×10^7 cells. The mouse was sacrificed by cervical dislocation, and its spleen was obtained. It was placed into the mesh (Sigma, USA) container, and the cells were separated. After making suspension of the spleen cells, the suspension was washed by centrifugation. The spleen cell solution was exposed to Tris-NH_4Cl solution (Tris 20.6g/L, NH_4Cl 8.3 g/L) to lyse red blood cells. After the completely separated antibody-producing cells were centrifuged for 5 min at 400g, they were washed two times with serum-free media and resuspended into the 10mℓ media. The lymph cells were counted by using a haemocytometer, and $1x10^8$ of lymphocytes were mixed with 1×10^7 of P3X63Ag 8.653 cells (10:1) in the serum-free media. The centrifugation was performed for 5 min at 400g.

<90> By using 50% (M/V) polyethylene glycol 1500 (Sigma, USA) pretreated at 37℃, 1mℓ solution was dropped to mix for 1 min. The fusion mixture solution produced in the above was diluted with serum-free RPMI1640 and centrifuged for 3 min at 400g. The cells were suspended in the 35mℓ of RPMI1640 selection media supplemented with 20% fetal bovine serum and HAT (100μM hypoxanthine, 0.4μM aminopterin, 16μM thymidine). 100㎕ of the suspension

solution was loaded to 96 well plates coated with feeder cells (macrophages separated from abdominal cavity using RPMI1640) one day before and cultured at 37℃, 5% CO_2. After 5 days, HAT selection media were replaced in 2 to 3 days intervals, and the cells were cultured for 14 days. After 14 days, subculture was performed by replacing RPMI1640 media supplemented with 20% fetal bovine serum and HT (media in which 0.4μM aminopterine is removed from HAT media).

<91>

<92> 4) Selection and separation of fusion cells producing each antibody

<93> The supernatant of the culture fused in the above was obtained, and the enzyme immunoassay was performed to investigate the production of the provided antigen-specific antibody (Fig. 1). The culture solution of fusion cells which represents the titer more than 4 times compared with negative control was selected and transferred to a 24-well culture plate and a 25 cm^2 culture flask.

<94>

<95> 5) Separation of immunoglobulin and determination of isotype

<96> Ascites obtained from the immunized mouse are mixed with protein G agarose (Invitrogen) and purification was carried out.

<97> Using 10% SDS-PAGE, purity of IgG was confirmed and then, dialysis with PBS (20% glycerol) was performed and stored at -70℃.

<98> Pristine 0.5 mℓ (Sigma, USA) was injected to mouse (Balb/c nu/nu, Charles River Diagnostics, Japan) intraperitoneally to increase frequency of peritoneal tumor and 7 days later, $5x10^5$ fusion cells was injected intraperitoneally to induce ascites production. 10-14 days later, antibodies were isolated from the ascites. Monoclonal isotypes were determined with Isotyping kit (Zymed Labomouseories Inc. USA). As a result, the antibodies against EMAPII turned out to be IgG1 (Fig. 2).

<99>

<100> Example 2. Cloning of anti-EMAPII antibody variable domains

<101> (1) cDNA synthesis from hybridoma

<102> Total RNA was prepared from 5 x 10^5 hybridoma cells. cDNA was synthesized with oligo dT primers and reverse transcriptase using protocol which is commercially available from BioAtla.

<103>

<104> (2) PCR amplification of variable domains of mouse IgG

<105> 1) Light chain variable domains

<106> Kappa light chain variable domains were amplified from cDNA using a set of mouse specific kappa primers and amplification protocol which are commercially available from BioAtla. The forward primers are designed to amplify the mouse light chain variable domains in combination with a kappa specific reverse primer. Negative controls were performed at the same time by adding H_2O instead of cDNA. Seven different primer combinations (K2, K3, K6, K7, K8, K9, and K11; forward primer mK2, mK3, mK6, mK7, mK9 and mK11 in Fig. 3) resulted in a PCR product of the expected size (~430bp) (Fig. 3). PCR products were gel purified, TOPO-TA cloned and sequenced (6 clones each).

<107>

<108> 2) Heavy chain variable domain

<109> Heavy chain variable domains were amplified from the above obtained cDNA using a set of mouse specific heavy chain primers and amplification protocol which are commercially available from BioAtla. The forward primers are designed to amplify the mouse heavy chain variable domains in combination with a mouse IgG1/2 specific reverse primer. Negative controls were performed at the same time by adding H_2O instead of cDNA. cDNA quality was checked by amplification of a 500 bp fragment of the mouse GAPDH.

<110> 7 different primer combinations (mH1 - mH7) resulted in a PCR product of the expected size (~450 bp) (Fig. 4). PCR products were gel purified, TOPO-TA cloned and sequenced (6 clones each).

<111>

<112> (3) Sequence analysis of PCR products after cloning using TOPO-TA vector

<113> The PCR products were TOPO TA cloned (Invitrogen TOPO TA vector) and

sequenced using M13 forward primer (gtaaaacgacggccag: SEQ ID NO.4).

<114>

<115> 1) Light chain variable domain

<116> Sequence analysis revealed that primer combinations mK2 and mK3 amplified the same light chain sequence (Fig. 5). The clones have a stop codon in the CDR3/Framework 4 region yielding a non productive V-J rearrangement. This sequence is commonly found in hybridomas made with fusion partners derived from the original MOPC-21 tumor.

<117> Sequence analysis (VectorNTI, Invitrogen) of the clones derived with primers mk6, mK7, mK8, mK9, and mK11 showed that the same light chain sequence was amplified (Fig. 6). The obtained clones have only a few amino acid differences at the amino terminus which are introduced by ambiguities in the PCR primers. The sequences were blasted against the mouse genome. The mouse germ line gene Vk19-17 was identified as closest match in the data base. This germ line gene encodes residues 1-95 of the target antibody (Fig. 6, top row of alignment). All clones have a Y49F mutation at position 49, probably introduced into the light chain gene by somatic hypermutation during B cell differentiation.

<118> In order to verify the N-terminus of the obtained light chain sequence, additional PCR reactions were performed with a forward primer annealing to the secretion signal of germ line gene Vk19-17 and a reverse primer specific for the CDR3 in the derived clones (Fig. 6, positions 89-97), or with the kappa specific reverse primer used in the initial PCR reactions.

LC primer sequence	mVk19-17_leadF: ATGGAGTCACAGATTCAGGTCTTTG
	BAP015_LC_CDR3_R: CCGAACGTGTACGGAGTACTATAATG
HC primer sequence	mVHJ558_F: CAGGTCCAGCTGCAGCAG
	BAP015_HV_CDR3_R: CCCCAGTAAGCAAACCGACTTG

<119>

<120>

<121> Both primer combinations resulted in a PCR product of the expected size (Fig. 7). The LC variable domain was amplified with 2 primer combinations

(lanes 1 and 2), and the HC variable domain was amplified using 4 primer combinations (lanes 3-6). Expected sizes of PCR products were ~ 330 bp (lanes 1, 3, 4) and ~500 bp (lanes 2, 5, 6), respectively. PCR products were gel purified, TOPO-TA cloned and sequenced (6 clones each). Both PCR reactions amplified the same light chain variable domain. The obtained DNA and deduced amino acid sequence is shown Fig. 8 (SEQ ID NO. 5). Only a single productive light chain sequence was amplified from the provided Hybridoma cell line. Mouse germ line gen Vk19-17 was identified as closest match. CDR residues are shown in bold.

<122>

<123> 2) Heavy variable domain

<124> Sequence analysis revealed that primer H4 amplified a non-productive rearranged heavy chain (Fig. 9). The identified sequence is derived from a non-productive V-D-J recombination originating probably from germline gene Vh14-3*2. Fragment D is out of frame creating a stop signal in the CDR 3 region.

<125> Primer combinations H1, H2, H3, H5, H6, and H7 amplified the same heavy chain variable domain, derived from germ line gene Vh1-4*1 (Fig. 10). All primer combinations amplify the same heavy chain variable domain, derived from germ line gene Vh1-4*1. Only sequence differences to Vh1-4*1 are shown. The sequence variations in the first 10 amino acids are caused by ambiguities in the PCR primers. Amino acid changes in individual clones (e.g. pos. 14, pos. 33, pos. 58, pos. 88 and pos. 98) are probably created during PCR.

<126> Additional PCR reactions with gene specific primers were performed in order to verify the obtained HC variable domain sequence. The resulting PCR products were TOPO-TA cloned and sequenced. All clones had the exact same sequence (Fig. 11, SEQ ID NO. 6). Only a single productive heavy chain sequence was amplified from the provided Hybridoma cell line. Mouse germ line gen Vh1-4*1 was identified as closest match. CDR residues are shown in bold.

<127>

<128> Example 3. Preparation of anti-EMAPII chimeric antibody

<129> (1) Cloning of anti-EMAPII chimeric antibody-Subcloning of Fab domain

of light chain in human kappa Fc region and of Fab domain of heavy chain in human Ig G1 Fc region

<130> The variable domains of the obtained murine(mouse) anti-EMAPII antibody were cloned into mammalian expression system pBAK2b which is commercially available from BioAtla.

<131> The light chain variable domain was fused in frame to a human kappa constant region; the heavy chain variable domain was fused in frame to a human IgG1 constant region. Both genes are preceded by a leader peptide for secretion of full length IgG1 antibodies into the medium.

<132> Five clones were sequenced to confirm the integrity and sequences of LC and HC reading frames in the expression vector. Three clones were selected for expression tests in CHO cells.

<133> Glycerol stocks of the three clones were prepared and endotoxin-free plasmid DNA was prepared for expression tests in CHO cells.

<134>

<135> (2) Quantification of chimeric IgG1 after transfection into CHO cells

<136> The plasmid DNA obtained as described above was transfected into CHO-S cells. One week before transfection, CHO-S cells (CD-CHO: Invitrogen, 10743-029) were transferred into monolayer culture in serum-supplemented DMEM (Dulbecco's Modified Eagle Medium: Invitrogen, 11965-092). One day before transfection, 0.4×10^5 cells were plated into 100㎕ serum-supplemented DMEM in a 96-well format for each transfection sample. A DNA-lipofectamine complex was prepared for each transfection sample. For this purpose, 0.2㎍ of DNA was diluted in 25㎕ of Opti-MEM reduced serum medium. 0.5㎕ of lipofectamine (lipofectamine 2000: Invitrogen, 11668-027) was diluted in 25㎕ of Opti-MEM reduced serum medium. The lipofectamine dilution was incubated at room temperature for 5 minutes. The diluted DNA and the diluted lipofectamine were mixed with each other, and then incubated at room temperature for 20 minutes. 50㎕ of the resulting DNA-lipofectamine complex was added to each well containing the cells and the medium, and the content of each well was lightly mixed. Then, the cells were incubated overnight in a 5% CO_2 incubator at 37℃. The medium in each well was removed by suction, and 100㎕ of serum-

supplemented DMEM was added to each well. 48 hours after transfection, the cell culture supernatant was collected, and the concentration of recombinant chimeric IgG in the cell culture supernatant was determined by ELISA. The recombinant chimeric IgG in the cell culture supernatant was captured using an anti-human Fc antibody immobilized on a plate. The bound chimeric IgG was detected using an anti-human IgG HRP conjugate and quantified using commercial human IgG as a standard.

<137> In brief, each well of a Nunc-Immuno Maxisorp 96-well plate (Nalge Nunc, 439454) was coated with 100㎕ of a coating solution containing 10㎍/㎕ of affinity-purified Fc-specific goat anti-human IgG (Sigma, 12136-1ml). The plate was sealed and incubated overnight at 4℃. Each well of the plate was washed with 200㎕ of washing buffer with stirring at 200 rpm at room temperature for 5 minutes. 200㎕ of blocking buffer was added to each well of the plate, and the plate was stirred at 200 rpm at room temperature for 1 hour. 100㎍ of purified human serum IgG (Invitrogen, 12000C) in 1㎕ of blocking buffer was added in duplicate to each well of the plate. 100㎕ of the supernatant resulting from the transfection process was added in duplicate to each well of the plate. Then, the plate was stirred at 200 rpm at room temperature for 1 hour. Each well of the plate was washed twice with 200㎕ of washing buffer at 200 rpm at room temperature for 5 minutes. 100㎕ of a 1:5000 dilution of HRP-conjugated affinity-purified goat anti-human antibody (Promega, W4031) in blocking buffer was added to each well of the plate. The plate was stirred at 200 rpm at room temperature for 1 hour, after which each well was washed three times with 200㎕ of washing buffer at 200 rpm at room temperature for 5 minutes. TMB substrate (Sigma) was added to each well, and then the plate was incubated at room temperature. 100㎕ of 1N HCl was added to each well to terminate the reaction. Then, each well was read at 450 nm.

<138>

<139> (3) Comparison of affinity of monoclonal antibody with chimeric antibody

<140> 1) Optimization of ELISA

<141> In order to identify the linear range for testing the antigen binding of the murine and chimeric clones, a matrix experiment was set up as follows:

<142> Wells of a 96 well plate were coated with 100μl of different concentrations of EMAPII antigen (10μg/ml, 5μg/ml, 2.5μg/ml, 1μg/ml, 0.5 μg/ml, 0.2μg/ml) and were incubated at 37℃ for 2 hours. The coated antigens were blocked using BSA and washed with washing buffer. Dilutions of the parental murine anti-EMAPII antibody (starting at 1 μg/ml, two-fold serial dilution) were added to the antigens and incubated for 2 hours. The mixture was washed three times and anti-IgG-HRP antibody was added to the mixture and incubated for 1 hour. The mixture was washed with washing buffer three times. The substrate was added to the mixture and maintained for 30 minutes. After developing, stop solution was added and detected absorbance at the wavelength of 450 nm (Fig 12). Bound antibody was detected with anti-mouse IgG-HRP conjugate. A concentration of 2.5μg/ml antigen was selected for testing the chimeric clones.

<143>

<144> 2) Comparison of affinity

<145> The ability of mouse/human chimeric antibody BAP015_1 to bind to the EMAPII antigen was determined by using the ELISA protocol provided by StemScience and the optimized conditions determined as described above. Bound chimeric antibody was detected with anti-human-IgG HRP conjugate (Fig. 13). All 3 clones tested bind to EMAPII. The binding curves of all 3 clones are very similar to each other and to the murine antibody.

<146> The murine EMAPII mAb reached 50% binding saturation at 20.9ng/ml (Fig. 13). The chimeric antibody reached 50% binding saturation at 21.5-30.2ng/ml. Taken all assay variations into account, the data indicate that the chimeric antibody binds with very similar affinity to the target EMAPII antigen as the original murine mAb.

<147>

<148> Example 4. Humanization of anti EMAPII chimeric antibody

<149> (1) Cloning of Fab domain of heavy and light chain (full length) in human library

<150> 1) Construction of humanized variable domains

<151> Double stranded DNA fragments coding for the light chain and heavy chain CDR sequences from chimeric clone BAP015_1 (SEQ ID NOs. 11 and 19) were combined with BioAtla proprietary pools of human frameworks. Full length variable domains were then cloned into BioAtla's proprietary mammalian expression system pBAK2.

<152> Light chain variable domains were cloned in frame with a secretion signal and a human kappa constant domain. Heavy chain variable domains were cloned in frame with a leader sequence and a human IgG1 constant domain. The library of humanized variants was frozen as glycerol stock.

<153>

<154> 2) Manufacturing of plasmid DNA after transformation of E. coli

<155> Aliquots of the humanized library were plated and single colonies arrayed into to 96well plates. Each plate also contained 3 wells with positive control (BAP015_1) and negative control (vector only). Cultures were grown over night and plasmid DNA was prepared for transfection.

<156>

<157> 3) Transfection of humanized library

<158> CHO S cells were seeded in 96-well plates and tranfected with mini-prepped DNA of the humanized clones. Specific method was the same as the transfection method as described in the above (example 3-(2)).

<159>

<160> 4) Culture and measurement of Ig G

<161> Cell culture supernatant was collected at 48 hours after transfection and IgG concentration for each humanized clone and the controls was determined using BioAtla ELISA protocol for quantification of human IgGs as described for the chimeric antibody (example 3-(2)).

<162>

<163> 5) Measurement of activity using ELISA - selection of candidate, screening of humanized library

<164> Binding of the humanized clones to antigen EMAPII was tested in parallel using the antigen and protocol provided by Stemsciences. Specific

method was the same as described in the above (example 3). Specific activity (affinity/quant) was calculated for each clone and compared to the average specific activity of the positive control (chimieric clone BAP015_1) on the same plate. Clones with low expression levels were filtered out for selecting the primary hits. Clones with low expression levels (lower than BAP015_1) and low binding activity were filtered out for selecting the primary hits (candidates having effects of significance are referred to as ' hit'). Data from plate BAP015_scr155 are shown as an example in Figs. 14 and 15. The top hits from each plate were selected for confirmation and screened again.

<165>

<166> (2) Comparison of affinity of candidate and analysis of protein sequence

<167> 1) Confirmation screen

<168> The top hits from each plate were re-arrayed, re-transfected into CHO-S and screened in duplicates as described above. The top 7 clones (BAP015hum01 -BAP015hum07; respectively, in order, a pair of one of light chain variable domain sequences(SEQ ID NOs. 12 to 18) and one of heavy chain variable domain sequences(SEQ ID NOs. 20 to 26)) were selected for additional confirmation screenings.

<169> Dilutions of each antibody (1:50 to 1:100 for antigen binding; 1:200 and 1:400 dilutions were used for quant ELISA) were assayed for antigen binding in duplicates. For comparison, the same experiment was performed using chimeric antibodies (BAP015-1, BAP015-2) and mouse monoclonal antibody (positive). Specific activity for each dilution was calculated and the average specific activity for each clone was shown in Fig. 16.

<170>

<171> 2) Sequencing

<172> The light and heavy chain variable domains of the top 7 clones were sequenced, and the deduced amino acid sequences were aligned and compared to each other and to the mouse variable domains in clone BAP015_1. Each of the 7 top hits has a unique light chain (Fig. 17). Hits BAP015-hum01 to 04 share

the same framework lsequence and hits BAP015-hum06 and 07 share the same framework 1 sequence. BAP015-hum02 to 06 share the same frame work 2 sequence. Clones BAP015-hum04 to 07 share the same frame work 3 sequence. An alignment of the heavy chain variable domains is shown in Fig. 17. Only 2 different heavy chains were identified in the top humanized hits. The light chain CDRs and the heavy chain CDRs are boxed. The top 7 humanized hits contain 7 unique light chains and 2 unique heavy chains.

【CLAIMS】

【Claim 1】

<174> A humanized anti-EMAPII (endothelial monocyte activating polypeptide II) antibody, comprising: (i) a light-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 12 to SEQ ID NO: 18; and (ii) a heavy-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 20 to SEQ ID NO: 26.

【Claim 2】

<175> The humanized anti-EMAPII antibody of claim 1, wherein the humanized anti-EMAPII antibody comprises a pair of a light-chain variable domain and a heavy-chain variable domain selected from the group consisting of a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 12, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 20; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 13, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 21; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 14, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 22; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 15, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 23; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 16, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 24; a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 17, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 25; and a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 18, and a heavy-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 26.

【Claim 3】

<176> The humanized anti-EMAPII antibody of claim 1, wherein the humanized anti-EMAPII antibody further comprises a constant domain derived from human IgG1.

【Claim 4】

<177> A pharmaceutical composition for preventing or treating a TNF-α-mediated disease or Alzheimer's disease, comprising a humanized anti-EMAPII antibody of any of claims 1 to 3.

【Claim 5】

<178> The pharmaceutical composition of claim 4, wherein the TNF-α-mediated disease is selected from the group consisting of adult respiratory distress syndrome; anorexia; cancer; chronic fatigue syndrome; graft versus host rejection; hyperalgesia; inflammatory bowel disease; neuroinflammatory diseases; ischemic/reperfusion injury, including cerebral ischemia; brain injury as a result of trauma, epilepsy, hemorrhage or stroke; diabetes; multiple sclerosis; ocular diseases; pain; pancreatitis; pulmonary fibrosis; rheumatoid arthritis, osteoarthritis, juvenile (rheumatoid) arthritis, seronegative polyarthritis, ankylosing spondylitis, Reiter's syndrome and reactive arthritis, psoriatic arthritis, enteropathic arthritis, polymyositis, dermatomyositis, scleroderma, systemic sclerosis, vasculitis, cerebral vasculitis, Sjogren's syndrome, rheumatic fever, polychondritis and polymyalgia rheumatica and giant cell arteritis; septic shock; side effects from radiation therapy; systemic lupus erythematous; temporal mandibular joint disease; and thyroiditis.

【Claim 6】

<179> A kit for quantification of EMAPII comprising a humanized anti-EMAPII antibody of any of claims 1-3.

【Claim 7】

<180> A nucleic acid molecule encoding a light-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 12 to SEQ ID NO: 18.

【Claim 8】

<181> A nucleic acid molecule encoding a heavy-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 20 to SEQ ID NO: 26.

【Claim 9】

<182> A recombinant vector comprising nucleic acid molecules encoding a light-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 12 to SEQ ID NO: 18 and/or heavy-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 20 to SEQ ID NO: 26.

【Claim 10】

<183> A transformed cell comprising the recombinant vector of claim 9.

【Claim 11】

<184> A method of producing a humanized anti-EMAPII antibody by culturing transformed cell of claim 10.

【Claim 12】

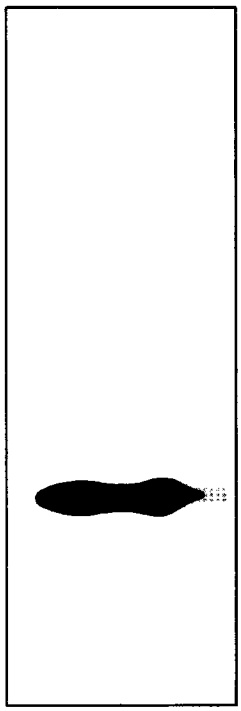
<185> A method of preventing or treating a TNF-α-mediated disease or Alzheimer's disease, comprising a step of administering a humanized anti-EMAPII antibody of any of claims 1 to 3 or a pharmaceutical composition of claim 4 or 5.

【Claim 13】

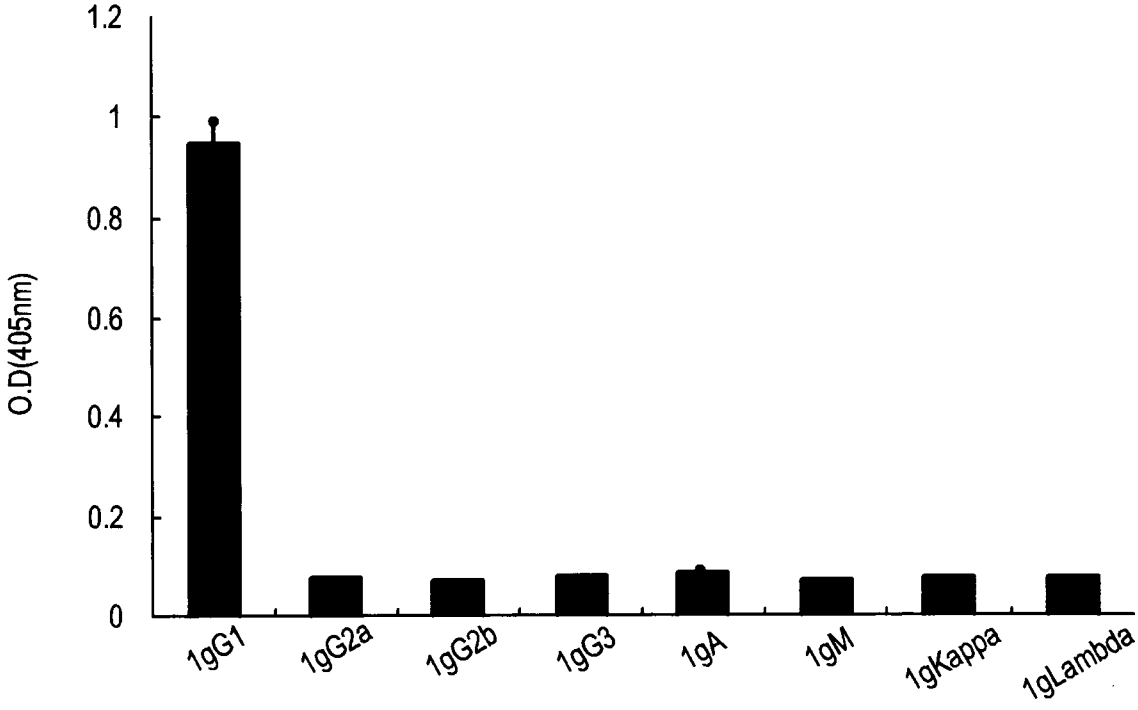
<186> A chimeric anti-EMAPII antibody comprising: a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 11; and a heavy-chain variable domain set forth in SEQ ID NO: 19.

【DRAWINGS】

【Figure 1】

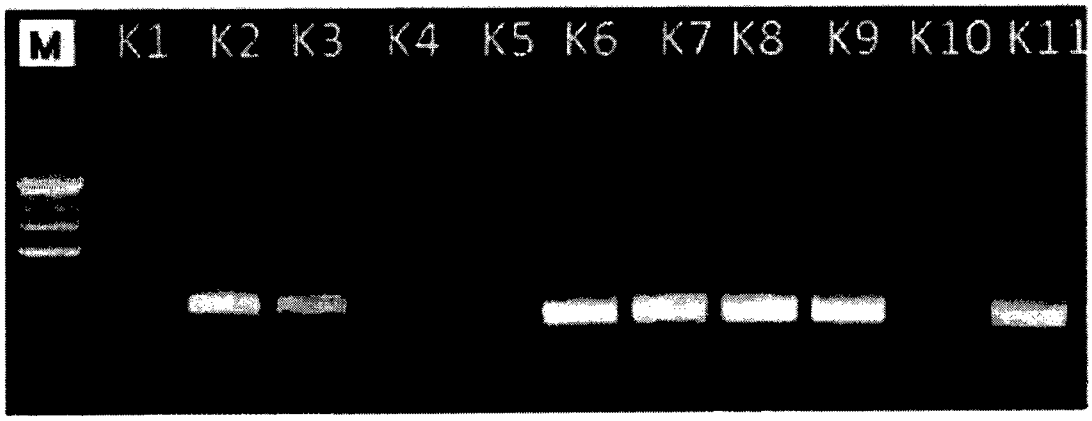


【Figure 2】



【Figure 3】

PCR products obtained with kappa specific combinations



M: DNA maker (smallest fragment 500 bp)

Lanes 1.11: PCR with primer combinations mK1-mL11

【Figure 4】

PCR products obtained with heavy chani specific primer combinations

M: DNA maker (smallest fragment 500 bp)

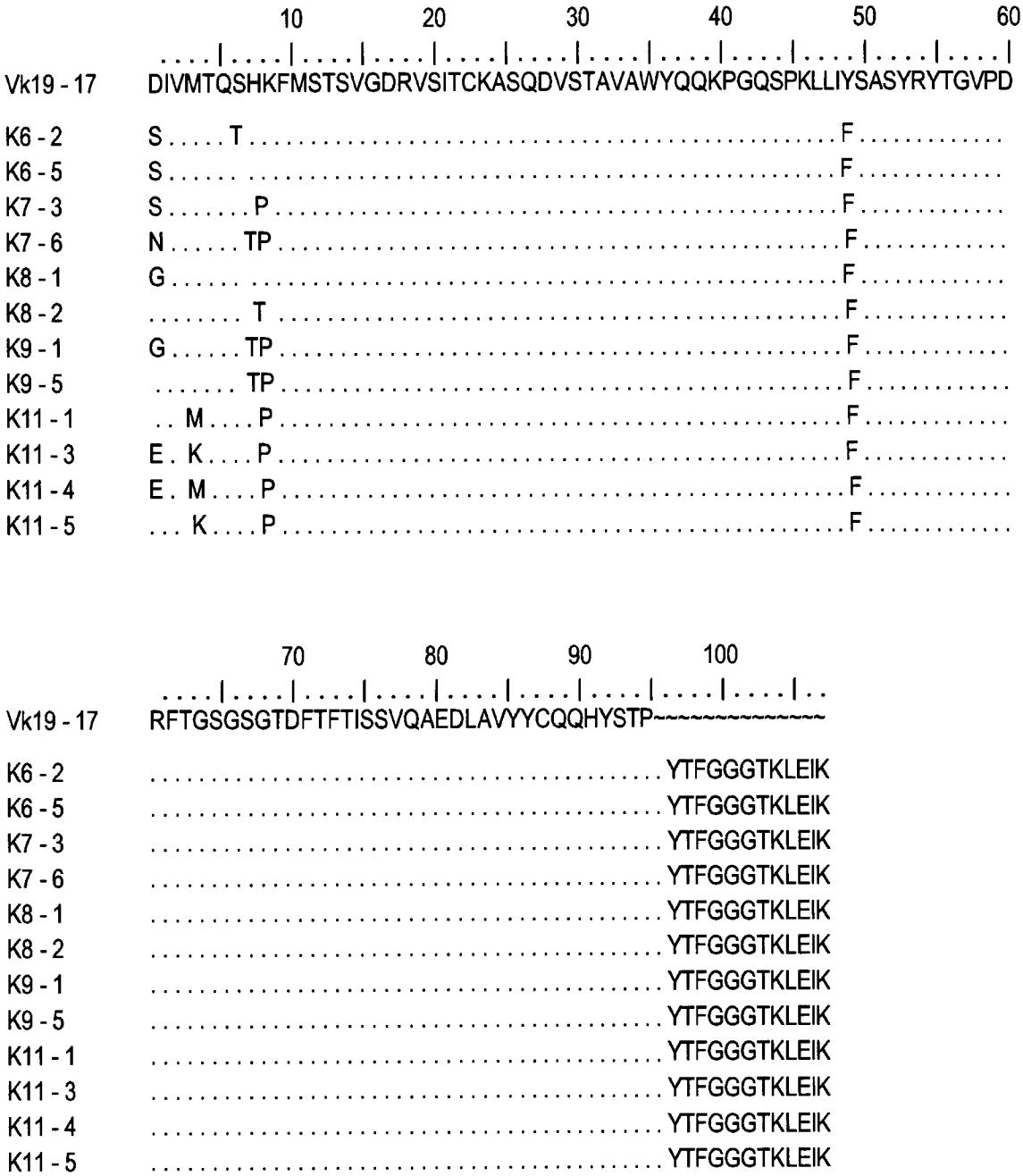
Lanes 1.7: PCR with primer combinations mH1-mH7

Lane 8: control PCR mouse GAPDH

【Figure 5】

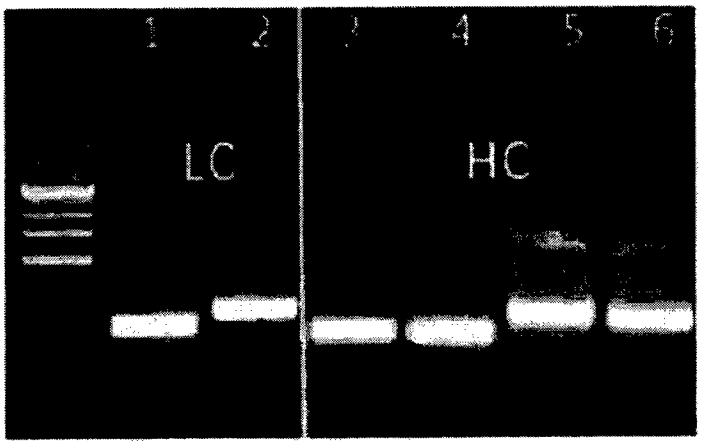
```
                    10        20        30        40        50        60
                    ....|....|....|....|....|....|....|....|....|....|....|
AAA39004            DIVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES

K2-1-T7             NIVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K2-2-T7             DIVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K2-3-T7              IVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K2-4-T7              IVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K2-5-T7             DIVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K2-6-T7             DIVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K3-2-T7             ENVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K3-4-T7             ENVLSQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K3-5-T7             ENVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES
K3-6-T7             ENVLTQSPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLES

                    70        80        90        100
                    ....|....|....|....|....|....|....|....|....
AAA39004            GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*

K2-1-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K2-2-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K2-3-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K2-4-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K2-5-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K2-6-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWE*
K3-2-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K3-4-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K3-5-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
K3-6-T7             GVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWK*
```

【Figure 6】


10 20 30 40 50 60
Vk19 - 17 DIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVPD
K6 - 2 S.....T.....F.....
K6 - 5 S.....F.....
K7 - 3 S.....P.....F.....
K7 - 6 N.....TP.....F.....
K8 - 1 G.....F.....
K8 - 2 ..... T .....F.....
K9 - 1 G.....TP.....F.....
K9 - 5 ..... TP.....F.....
K11 - 1 .. M....P.....F.....
K11 - 3 E. K....P.....F.....
K11 - 4 E. M....P.....F.....
K11 - 5 ... K....P.....F.....
70 80 90 100
Vk19 - 17 RFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYSTP~~~~~~~~~~~~~~
K6 - 2 .....YTFGGGTKLEIK
K6 - 5 .....YTFGGGTKLEIK
K7 - 3 .....YTFGGGTKLEIK
K7 - 6 .....YTFGGGTKLEIK
K8 - 1 .....YTFGGGTKLEIK
K8 - 2 .....YTFGGGTKLEIK
K9 - 1 .....YTFGGGTKLEIK
K9 - 5 .....YTFGGGTKLEIK
K11 - 1 .....YTFGGGTKLEIK
K11 - 3 .....YTFGGGTKLEIK
K11 - 4 .....YTFGGGTKLEIK
K11 - 5 .....YTFGGGTKLEIK

【Figure 7】

PCR of variable domains with gene specific primers



M: DNA maker (smallest fragment 500 bp)

Lane 1: LC PCR product primers annealing to secretion signal and CDR3

Lane 2: LC PCR product primers annealing to secretion signal and kappa constant region

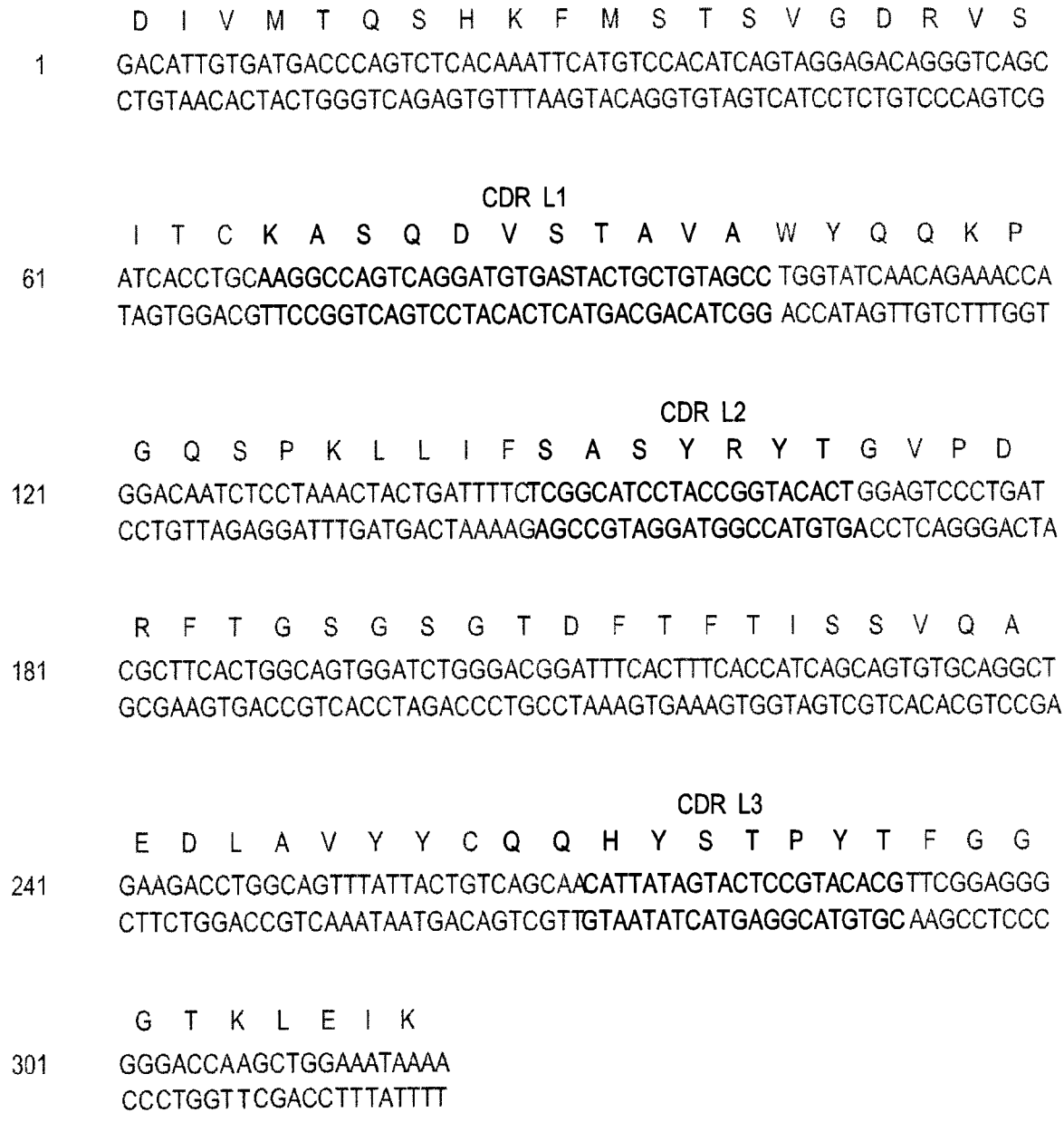
Lane 3: HC PCR product primers annealing to secretion signal and CD3

Lane 4: HC PCR product primers annealing to 5' and of variable domain and CDR3

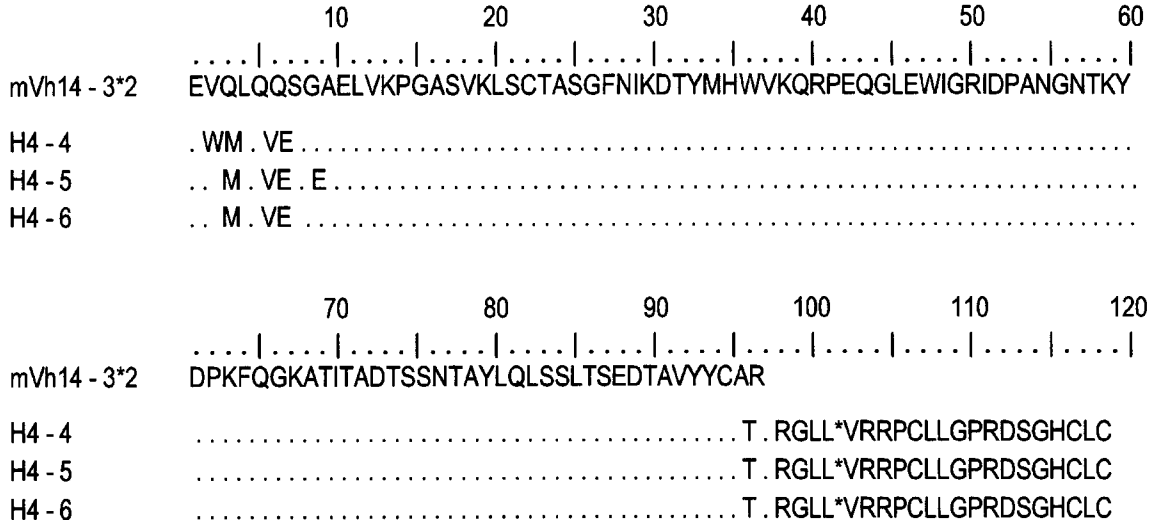
Lane 5: HC PCR product primers annealing to secretion signal and HC constant domain

Lane 6: HC PCR product primers annealing to 5' and of variable domain and HC constant domain

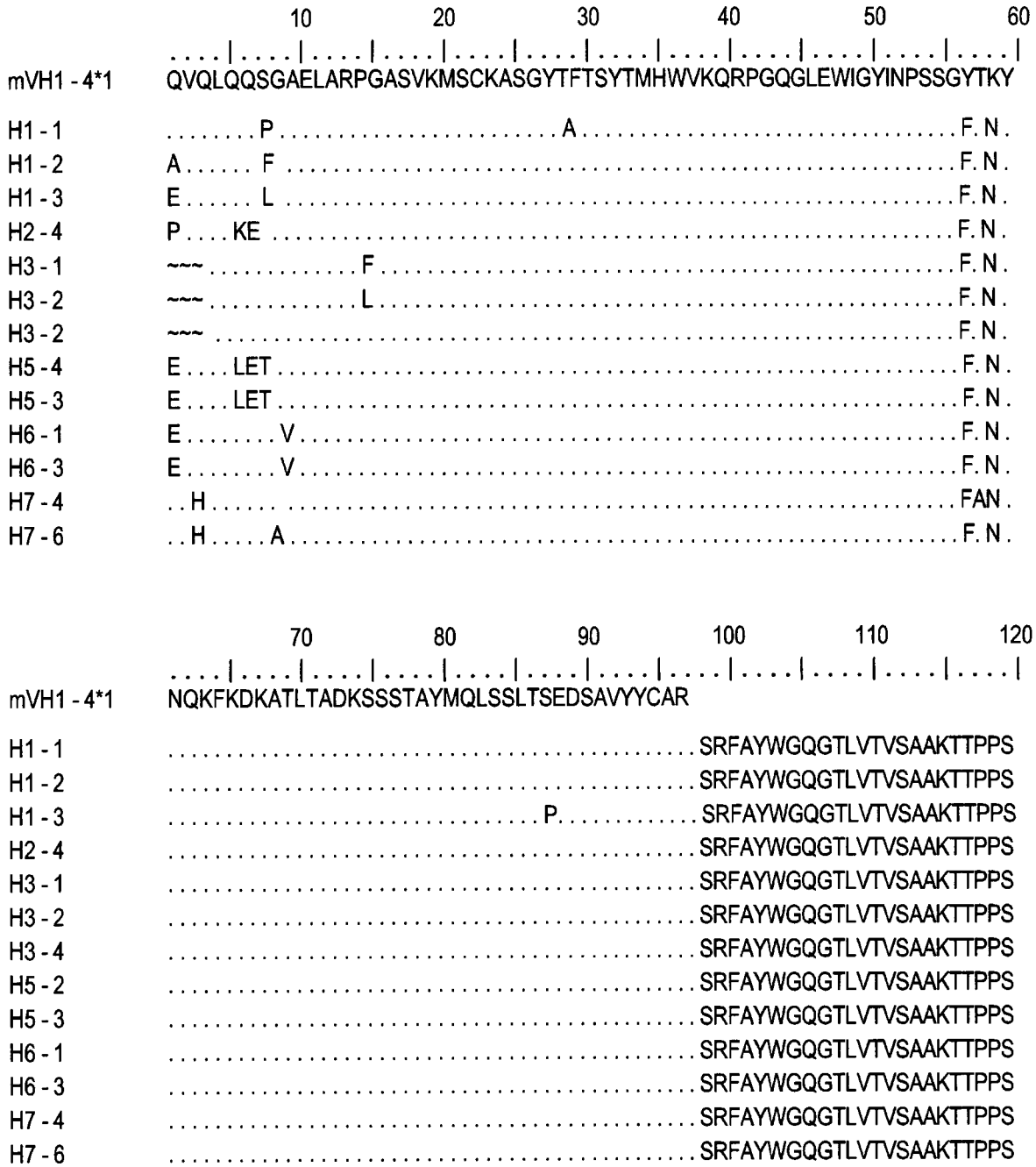
【Figure 8】


D I V M T Q S H K F M S T S V G D R V S
1 GACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGC
CTGTAACACTACTGGGTCAGAGTGTTTAAGTACAGGTGTAGTCATCCTCTGTCCCAGTCG
CDR L1
I T C K A S Q D V S T A V A W Y Q Q K P
61 ATCACCTGCAAGGCCAGTCAGGATGTGASTACTGCTGTAGCC TGGTATCAACAGAAACCA
TAGTGGACGTTCCGGTCAGTCCTACACTCATGACGACATCGG ACCATAGTTGTCTTTGGT
CDR L2
G Q S P K L L I F S A S Y R Y T G V P D
121 GGACAATCTCCTAAACTACTGATTTTCTCGGCATCCTACCGGTACACT GGAGTCCCTGAT
CCTGTTAGAGGATTTGATGACTAAAAGAGCCGTAGGATGGCCATGTGACCTCAGGGACTA
R F T G S G S G T D F T F T I S S V Q A
181 CGCTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATCAGCAGTGTGCAGGCT
GCGAAGTGACCGTCACCTAGACCCTGCCTAAAGTGAAAGTGGTAGTCGTCACACGTCCGA
CDR L3
E D L A V Y Y C Q Q H Y S T P Y T F G G
241 GAAGACCTGGCAGTTTATTACTGTCAGCAACATTATAGTACTCCGTACACGTTCGGAGGG
CTTCTGGACCGTCAAATAATGACAGTCGTTGTAATATCATGAGGCATGTGC AAGCCTCCC
G T K L E I K
301 GGGACCAAGCTGGAAATAAAA
CCCTGGTTCGACCTTTATTTT

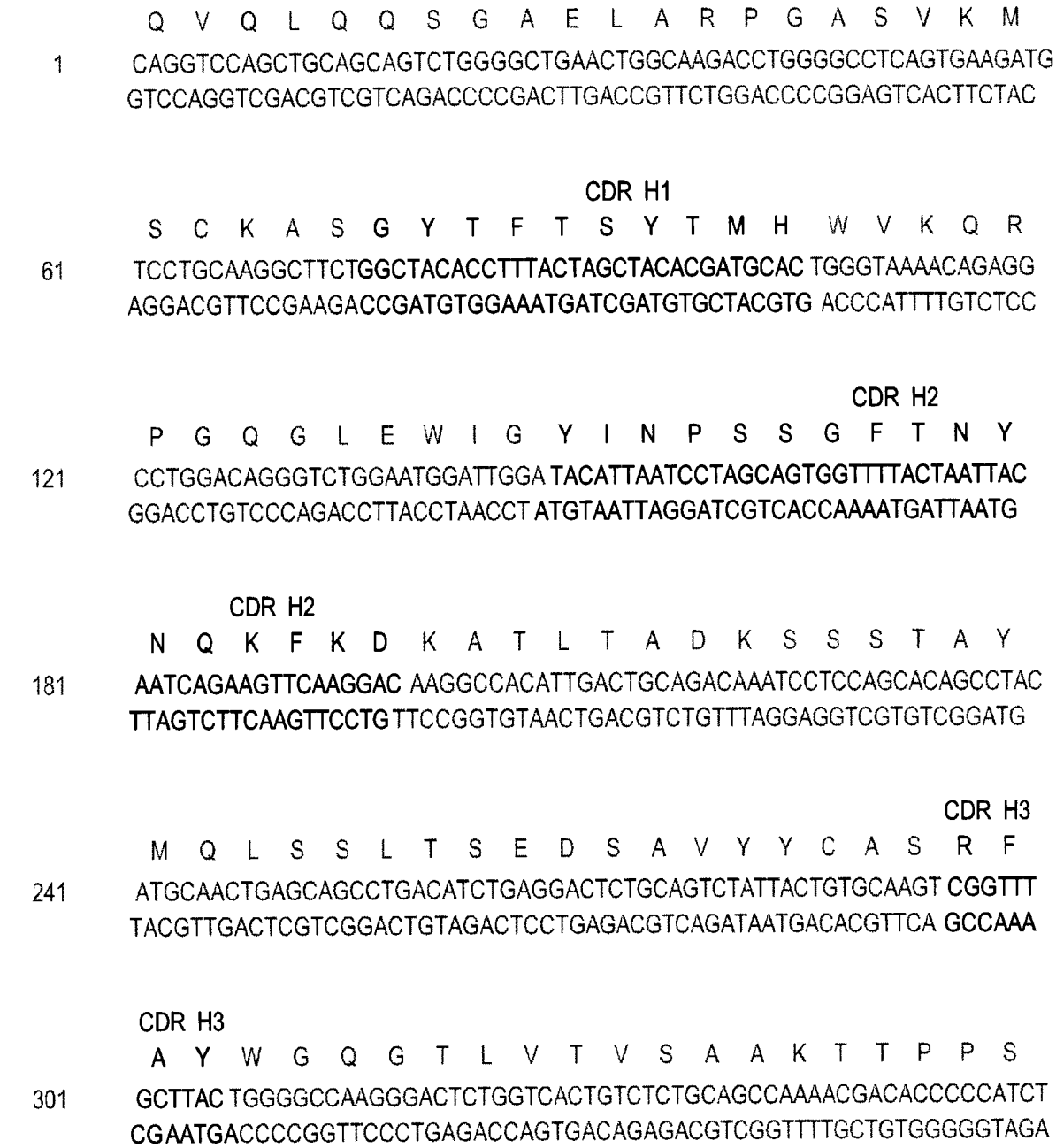
【Figure 9】


10 20 30 40 50 60
mVh14 - 3*2 EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRPEQGLEWIGRIDPANGNTKY
H4 - 4 . WM . VE
H4 - 5 . . M . VE . E
H4 - 6 . . M . VE
70 80 90 100 110 120
mVh14 - 3*2 DPKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCAR
H4 - 4 T . RGLL*VRRPCLLGPRDSGHCLC
H4 - 5 T . RGLL*VRRPCLLGPRDSGHCLC
H4 - 6 T . RGLL*VRRPCLLGPRDSGHCLC

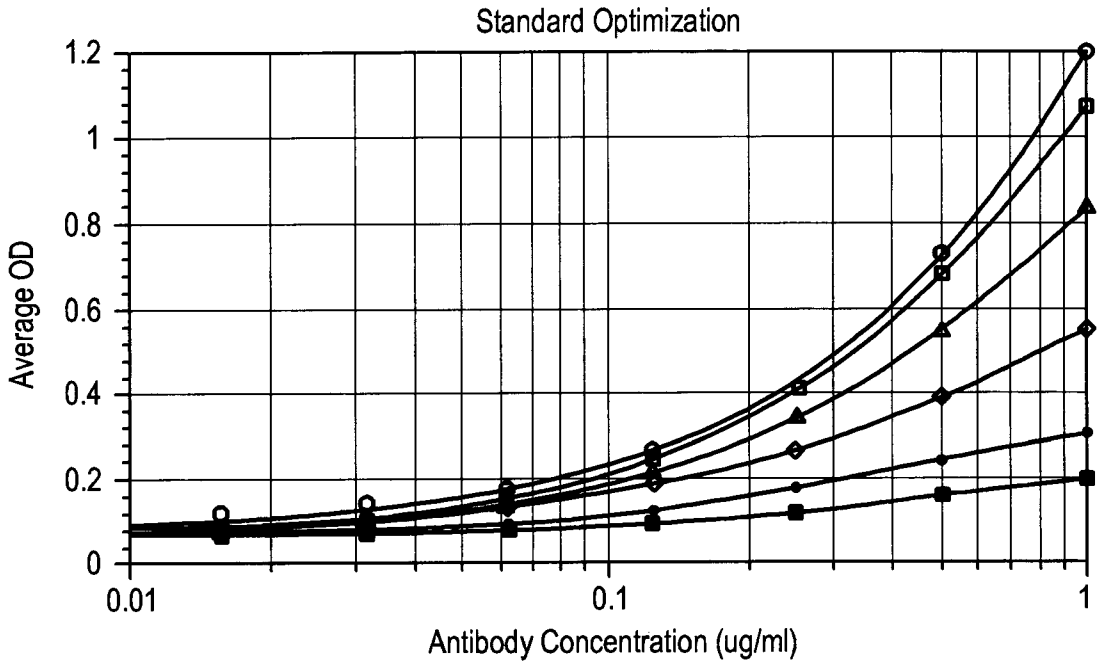
【Figure 10】


10 20 30 40 50 60
mVH1-4*1 QVQLQQSGAELARPGASVKMSCKASGYTFTSYTMHWVKQRPGQGLEWIGYINPSSGYTKY
H1-1 .....P.....A.....F.N.
H1-2 A.....F.....F.N.
H1-3 E.....L.....F.N.
H2-4 P....KE.....F.N.
H3-1 ~~~.....F.....F.N.
H3-2 ~~~.....L.....F.N.
H3-2 ~~~.....F.N.
H5-4 E....LET.....F.N.
H5-3 E....LET.....F.N.
H6-1 E.....V.....F.N.
H6-3 E.....V.....F.N.
H7-4 ..H.....FAN.
H7-6 ..H.....A.....F.N.
70 80 90 100 110 120
mVH1-4*1 NQKFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCAR
H1-1 .....SRFAYWGQGTLVTVSAAKTTPPS
H1-2 .....SRFAYWGQGTLVTVSAAKTTPPS
H1-3 .....P.....SRFAYWGQGTLVTVSAAKTTPPS
H2-4 .....SRFAYWGQGTLVTVSAAKTTPPS
H3-1 .....SRFAYWGQGTLVTVSAAKTTPPS
H3-2 .....SRFAYWGQGTLVTVSAAKTTPPS
H3-4 .....SRFAYWGQGTLVTVSAAKTTPPS
H5-2 .....SRFAYWGQGTLVTVSAAKTTPPS
H5-3 .....SRFAYWGQGTLVTVSAAKTTPPS
H6-1 .....SRFAYWGQGTLVTVSAAKTTPPS
H6-3 .....SRFAYWGQGTLVTVSAAKTTPPS
H7-4 .....SRFAYWGQGTLVTVSAAKTTPPS
H7-6 .....SRFAYWGQGTLVTVSAAKTTPPS

【Figure 11】


Q V Q L Q Q S G A E L A R P G A S V K M
1 CAGGTCCAGCTGCAGCAGTCTGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATG
GTCCAGGTCGACGTCGTCAGACCCCGACTTGACCGTTCTGGACCCCGGAGTCACTTCTAC
CDR H1
S C K A S G Y T F T S Y T M H W V K Q R
61 TCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACACGATGCAC TGGGTAAAACAGAGG
AGGACGTTCCGAAGACCGATGTGGAAATGATCGATGTGCTACGTG ACCCATTTTGTCTCC
CDR H2
P G Q G L E W I G Y I N P S S G F T N Y
121 CCTGGACAGGGTCTGGAATGGATTGGA TACATTAATCCTAGCAGTGGTTTTACTAATTAC
GGACCTGTCCCAGACCTTACCTAACCT ATGTAATTAGGATCGTCACCAAAATGATTAATG
CDR H2
N Q K F K D K A T L T A D K S S S T A Y
181 AATCAGAAGTTCAAGGAC AAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTAC
TTAGTCTTCAAGTTCCTG TTCCGGTGTAACTGACGTCTGTTTAGGAGGTCGTGTCGGATG
CDR H3
M Q L S S L T S E D S A V Y Y C A S R F
241 ATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGT CGGTTT
TACGTTGACTCGTCGGACTGTAGACTCCTGAGACGTCAGATAATGACACGTTCA GCCAAA
CDR H3
A Y W G Q G T L V T V S A A K T T P P S
301 GCTTAC TGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCCATCT
CGAATGACCCCGGTTCCCTGAGACCAGTGACAGAGACGTCGGTTTTGCTGTGGGGGTAGA

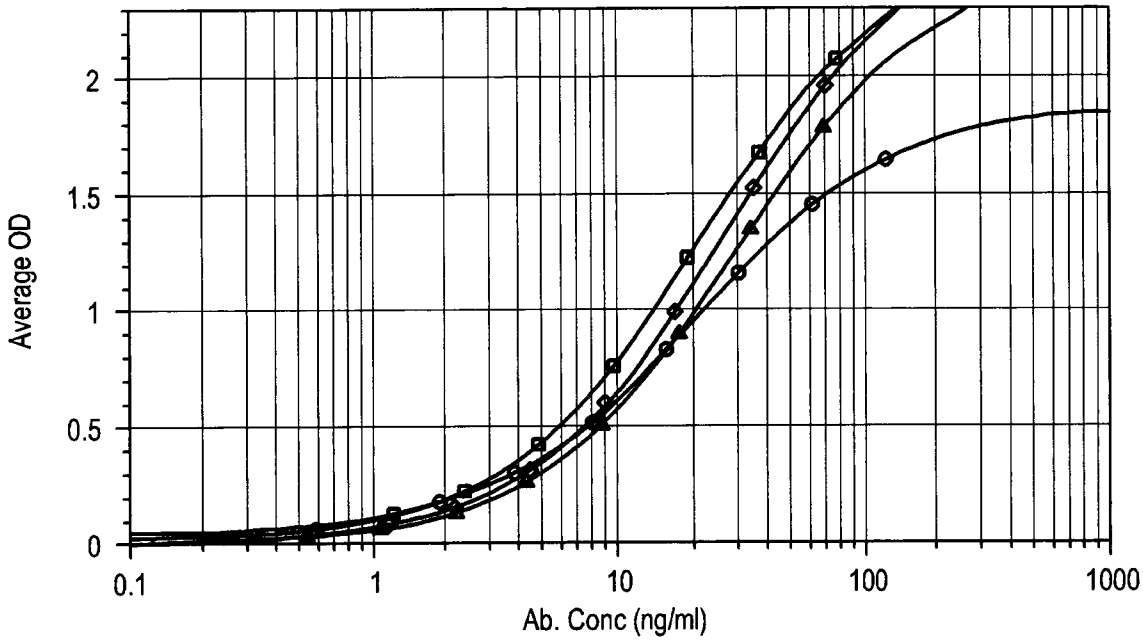
【Figure 12】



i - P Fit: y = (A - D) / (1 + (x / c) ^B) + D:	A	B	C	D	R^2
o 10ug/ml P43 Ag (Standards: Conc v	0.0681	0.91	7.19	8	0.999
□ 0.2ug/ml P43 Ag (0.2 ug/ml: Conc	0.0716	1.56	0.424	0.227	0.897
△ 0.5ug/ml P43 Ag (std5: Concentra	0.0647	1.23	0.422	0.385	0.994
◇ 1ug/ml P43 Ag (std4: Concentrati	0.0609	0.811	2.39	1.55	0.995
• 2.5ug/ml P43 Ag (std3: Concentra	0.0621	1.02	1.35	1.87	0.999
■ 5ug/ml P43 Ag (std2: Concentrati	0.0624	1.03	1.59	2.69	1

Curve Fit Option - Fixed Weight Value

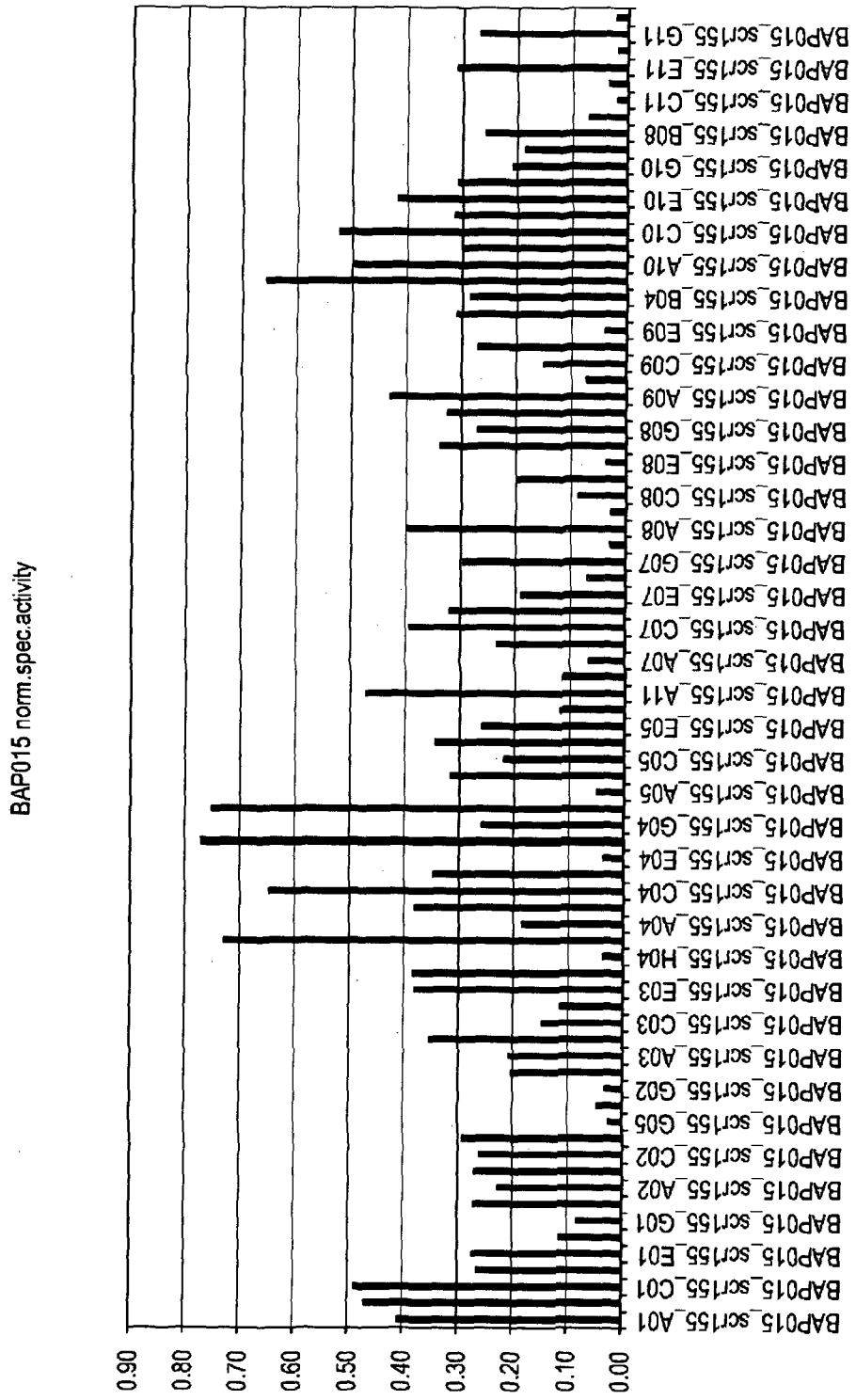
【Figure 13】



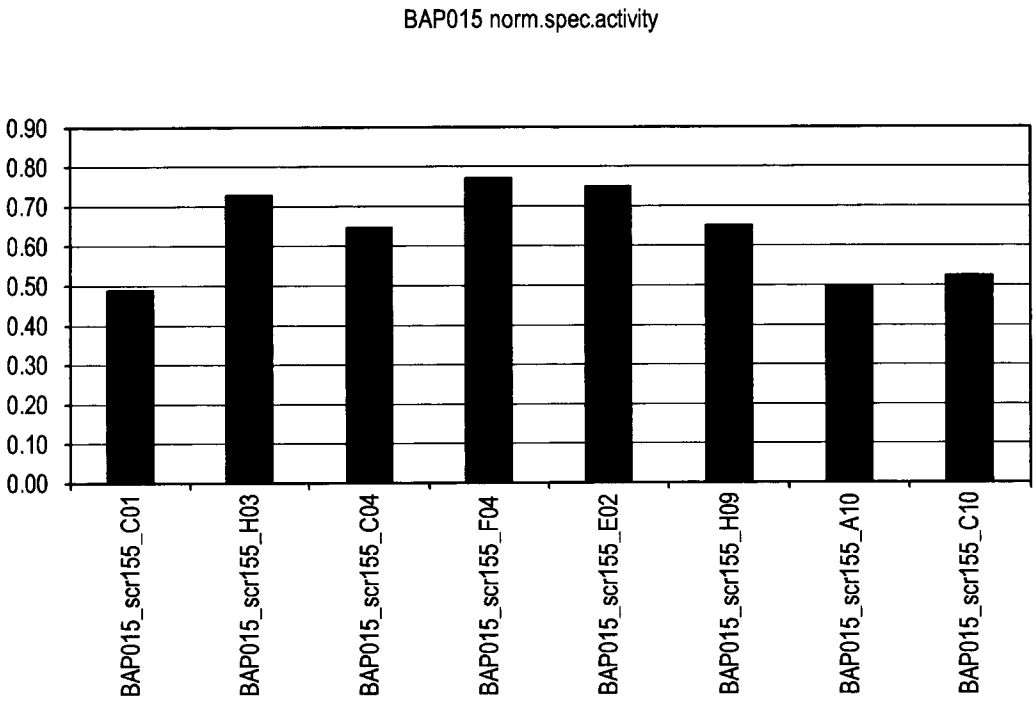
4 - P Fit: y = (A - D) / (1 + (x/C) ^B) + D:	A	B	C	D	R^2
o Mouse (murine anti-P43: ng/ml vs AvgOD)	0.0317	1.07	20.9	1.88	1
◇ Chimera 3 (MAP015-1/3: ng/ml vs AvgOD)	-0.00162	1.12	27.8	2.66	1
△ Chimera 2 (MAP015-1/2: ng/ml vs AvgOD)	-0.00536	1.11	30.2	2.49	1
□ Chimera 1 (MAP015-1/1: ng/ml vs AvgOD)	0.0108	1.11	21.5	2.57	1

Curve Fit Option - Fixed Weight Value

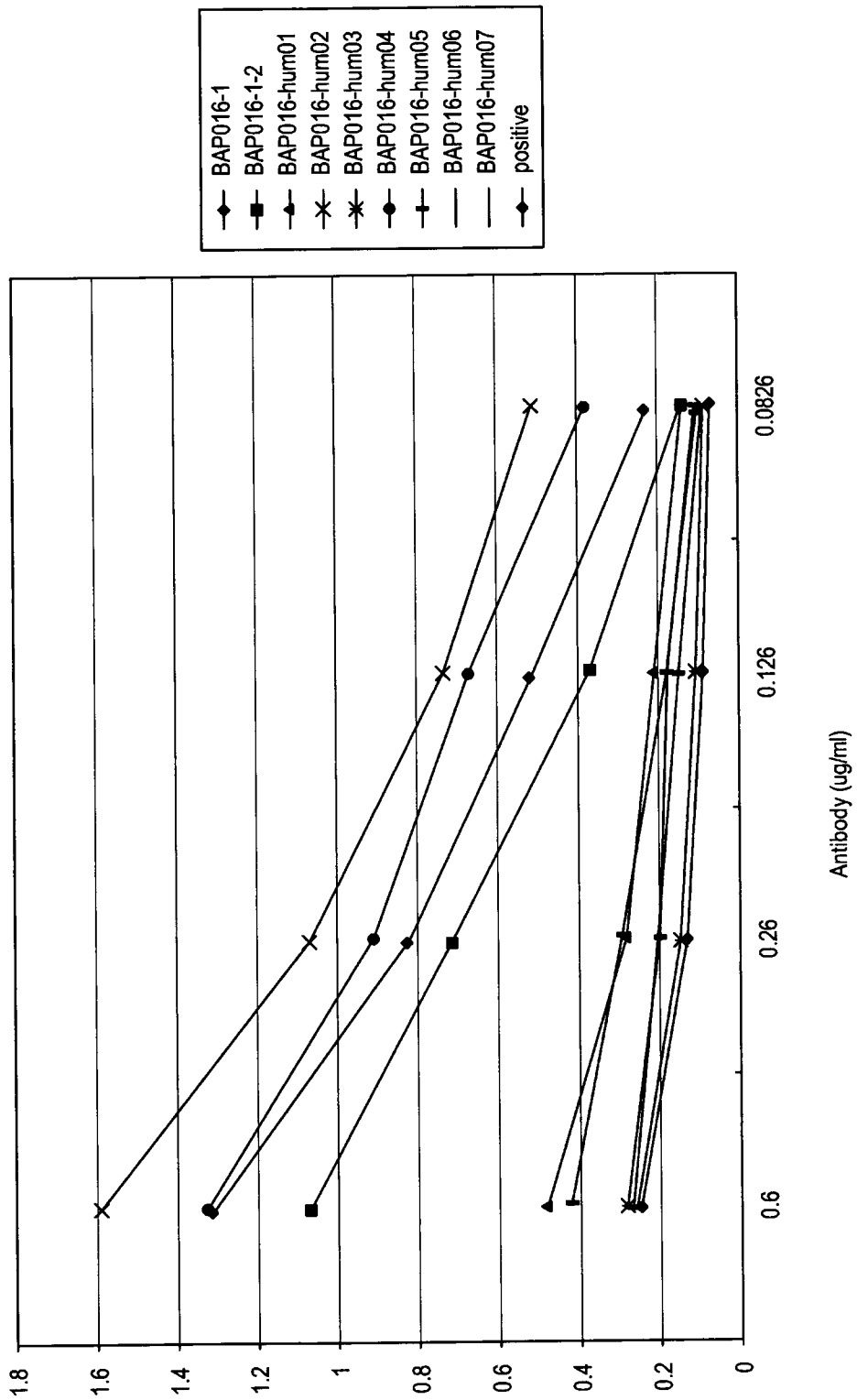
【Figure 14】


BAP015 nom.spec.activity
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
BAP015_scrl55_A01
BAP015_scrl55_C01
BAP015_scrl55_E01
BAP015_scrl55_G01
BAP015_scrl55_A02
BAP015_scrl55_C02
BAP015_scrl55_G02
BAP015_scrl55_G05
BAP015_scrl55_A03
BAP015_scrl55_C03
BAP015_scrl55_E03
BAP015_scrl55_H04
BAP015_scrl55_A04
BAP015_scrl55_C04
BAP015_scrl55_E04
BAP015_scrl55_G04
BAP015_scrl55_A05
BAP015_scrl55_C05
BAP015_scrl55_E05
BAP015_scrl55_A11
BAP015_scrl55_A07
BAP015_scrl55_C07
BAP015_scrl55_E07
BAP015_scrl55_G07
BAP015_scrl55_A08
BAP015_scrl55_C08
BAP015_scrl55_E08
BAP015_scrl55_G08
BAP015_scrl55_A09
BAP015_scrl55_C09
BAP015_scrl55_E09
BAP015_scrl55_B04
BAP015_scrl55_A10
BAP015_scrl55_C10
BAP015_scrl55_E10
BAP015_scrl55_G10
BAP015_scrl55_B08
BAP015_scrl55_C11
BAP015_scrl55_E11
BAP015_scrl55_G11

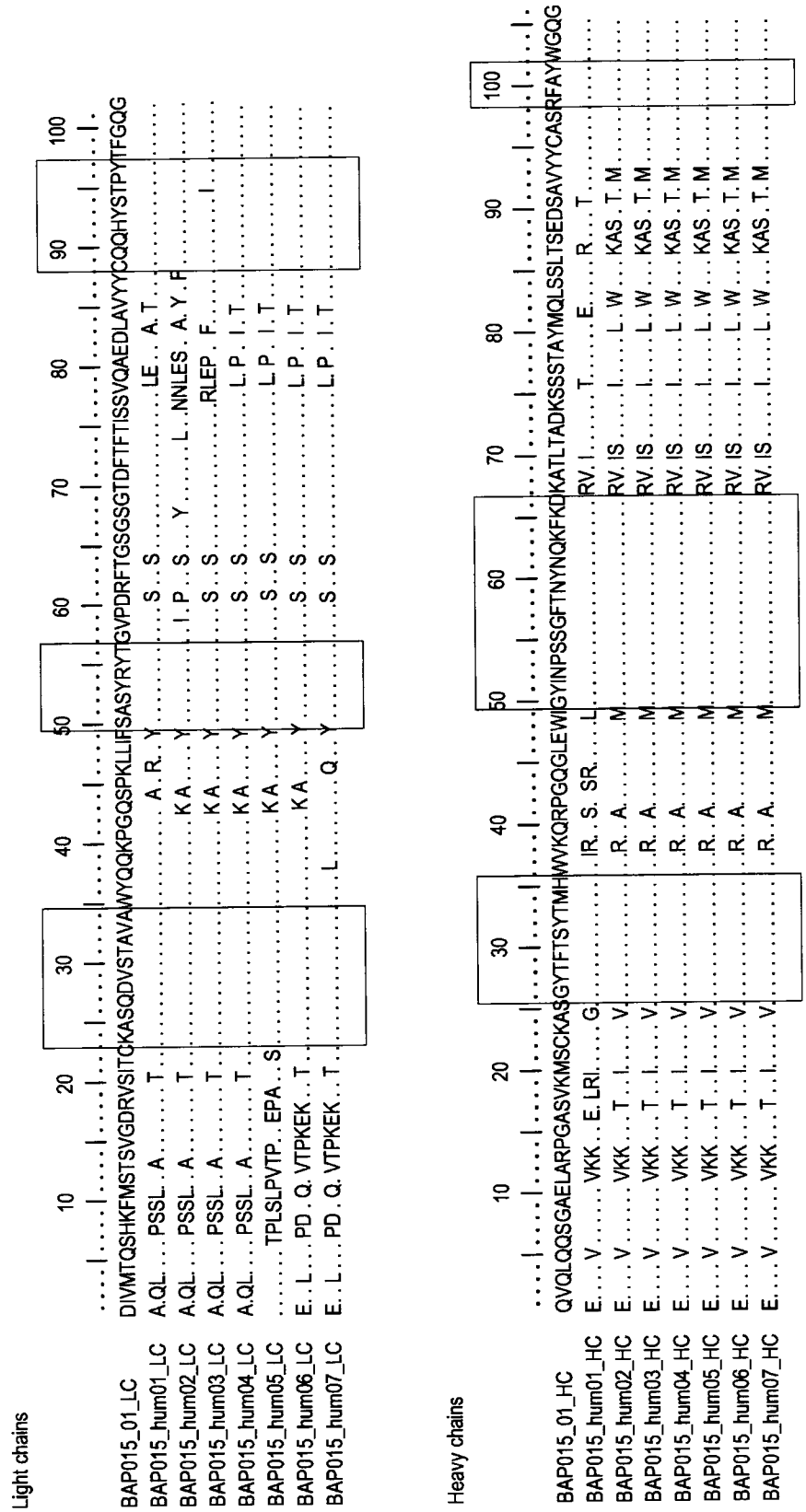
【Figure 15】


BAP015 norm.spec.activity
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
BAP015_scr155_C01
BAP015_scr155_H03
BAP015_scr155_C04
BAP015_scr155_F04
BAP015_scr155_E02
BAP015_scr155_H09
BAP015_scr155_A10
BAP015_scr155_C10

【Figure 16】


BAP016-1
BAP016-1-2
BAP016-hum01
BAP016-hum02
BAP016-hum03
BAP016-hum04
BAP016-hum05
BAP016-hum06
BAP016-hum07
positive
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.0826
0.126
0.26
0.6
Antibody (ug/ml)

【Figure 17】



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/005203

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/18(2006.01)i, A61K 39/395(2006.01)i, C12N 15/13(2006.01)i, G01N 33/53(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 16/18; C40B 40/10; C40B 40/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: endothelial monocyte activating polypeptide II, antibody, TNF-alpha-mediated disease, Alzheimer's disease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011-0028349 A1 (DAVE, J. R., et al.) 3 February 2011 See the whole document.	1-7,9-11
A	DEININGER, M. H. et al. Aberrant Neuronal and Paracellular Deposition of Endostatin in Brains of Patients with Alzheimer's Disease. The Journal of Neuroscience. 2002. Vol. 22, No. 24, pp. 10621-10626 See the whole document.	1-7,9-11
A	SCHLUESENER, H. J. et al. Localization of Endothelial-Monocyte-Activating Polypeptide II (EMAP II), a Novel Proinflammatory Cytokine, to Lesions of Experimental Autoimmune Encephalomyelitis, Neuritis and Uveitis: Expression by Monocytes and Activated Microglial Cells. Glia. 1997. Vol. 20, Issue 4, pp. 365-372 See the whole document.	1-7,9-11
A	ZHANG, Z. et al. Microglia activation in rat spinal cord by systemic injection of TLR3 and TLR7/8 agonists. Journal of Neuroimmunology. 2005, Vol. 164, Issues 1-2, pp. 154-160 See the whole document.	1-7,9-11

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 JUNE 2012 (12.06.2012)	**13 JUNE 2012 (13.06.2012)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
Kam Yoolim
Telephone No. 82-42-481-8741

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/005203

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG, Z. et al. Early infiltration of CD8+ macrophages/microglia to lesions of rat traumatic brain injury. Neuroscience. 2006. Vol. 141, Issue 2, pp. 637-644 See the whole document.	1-7,9-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/005203

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. a sequence listing filed or furnished

- ☐ on paper
- ☒ in electronic form

b. time of filing or furnishing

- ☒ contained in the international application as filed
- ☐ filed together with the international application in electronic form
- ☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/005203

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 12
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 12 pertains to method for treatment of the human body by surgery or therapy, as well as diagnostic method, and thus relates to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- Groups 1-14 (claims 1-11) based on the number of sequences related to a light-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 12 to 18 (claims 1-7, 9-11 (all partial)) and the number of sequences related to a heavy-chain variable domain having an amino acid sequence set forth in any one of SEQ ID NO: 20 to 26 (claims 1-6, 8-11 (all partial)); and
- Group 15 (claim 13) related to a light-chain variable domain having an amino acid sequence set forth in SEQ ID NO: 11 and a heavy-chain variable domain set forth in SEQ ID NO: 19.

Inventions belonging to Groups 1-15 do not share a single general inventive concept linking the 15 groups together within the meaning of PCT Rule 13.1.

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 9-11 partially related to SEQ ID NO: 12

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2011/005203

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011-0028349 A1	03.02.2011	US 2009-0068691 A1	12.03.2009
		US 7799536 B2	21.09.2010
		WO 2007-136617 A2	29.11.2007
		WO 2007-136617 A3	02.05.2008