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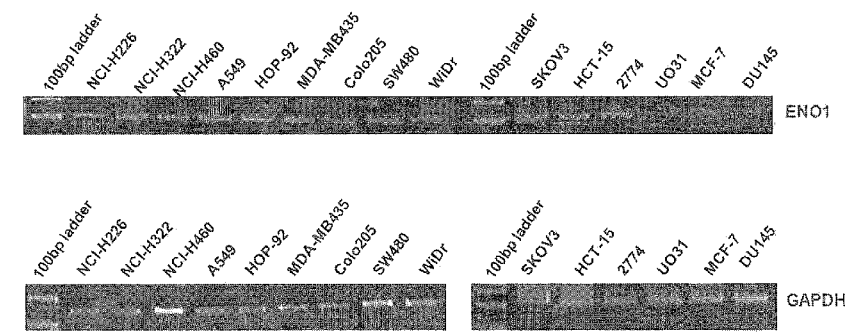
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(54) Title: ENO1-SPECIFIC HUMAN ANTIBODY

Fig. 1



(57) Abstract: The present invention relates to an alpha-endolase (ENO1) -specific human antibody, and more particularly to a human antibody including a complementarity determining region (CDR) and a framework region (FR) derived from a human antibody specifically bound to ENO1. The ENO1-specific human antibody expressed in the various kinds of cancer cells of the present invention may be used in diagnosis of the cancer, classification of the disease, visualization, treatment, and prognostic evaluation.

【DESCRIPTION】**【Invention Title】****ENO1-SPECIFIC HUMAN ANTIBODY**5 **【Technical Field】**

The present disclosure relates to a alpha-endolase (ENO1)-specific human antibody.

【Background Art】

10 It is known that enolase has α , β , and γ homologues. α -enolase is present in most tissues, but particularly predominant in early embryonic tissues. The α -enolase (or ENO1) and MBP-1 (c-myc binding protein) are derived from single genes by alternative use in translational starting
15 sites.

ENO1 is a cytoplasmic glycolytic enzyme which is significant in formation of phosphoenolpyruvate. It has been reported that ENO1, a major enzyme in glycolysis, is overexpressed in 18 kinds of cancer cells (Altenberg B,
20 Greulich KO. *enomics* 84:1014-1020, 2004). The immunogenicity of ENO1 in cancer cells is identified by Western blot and ELISA using recombinant proteins (Ping H et al., *Can Sci.* 98:1234-1240, 2007). In addition, it is confirmed by immunohistochemical staining (IHC staining) that ENO1 is
25 overexpressed in tumor tissues of non-small cell lung cancer

(NSCLC) patients, and by FACS that ENO1 is expressed on the surface of lung cancer cells. It is expected that as the expression of ENO1 is enhanced on the surface of cancer cells, autoantibody reaction is induced as a result of
5 autoantigenicity. As a reaction to up-regulation of ENO1 expression, the fibrinolytic system process is significantly accelerated. The resulting increase in local fibrinolysis is considered to contribute to invasion and metastasis of cancer cells. In the case of NSCLC patients, the results are
10 identical in the fact that patients with III/IV stage cancer tend to have more autoantibody dominance in autoantibody against ENO1 than those with I/II stage cancer. However, because the expression level of ENO1 in patients without autoantibody is also increased, ENO1 expression itself is not
15 sufficient for autoantibody production. Thus, studies on more specific mechanisms associated with autoantibody reaction are needed.

Thus, the present inventors have selected 6 kinds of human antibodies specifically bound to ENO1 expressing on the
20 surface of various kinds of cancer cell lines, confirmed that the human antibody has binding capacity similar to those of the conventional nonhuman-derived antibodies and that the human antibody of the present invention may be useful for diagnosis, prevention, and treatment of cancers, and have made
25 the present invention.

【Disclosure】**【Technical Problem】**

One object of the present invention is to provide an
5 ENO1-specific human antibody.

Another object of the present invention is to provide a
polynucleotide encoding a heavy chain of the human antibody or
a fragment thereof, and an expression vector including the
polynucleotide and a constant region of human heavy chain.

10 Still another object of the present invention is to
provide a polynucleotide encoding a light chain of the human
antibody or a fragment thereof, and an expression vector
including the polynucleotide and a constant region of human
light chain.

15 Even another object of the present invention is to
provide a transformant prepared by introducing an expression
vector including a polynucleotide encoding the heavy chain of
the human antibody or an immunologically active fragment
thereof into a host cell.

20 Yet another object of the present invention is to provide
a transformant prepared by introducing an expression vector
including a polynucleotide encoding the light chain of the
human antibody or an immunologically active fragment thereof
into a host cell.

25 Further another object of the present invention is to

provide a transformant prepared by introducing an expression vector including a polynucleotide encoding the heavy chain of the human antibody or a fragment thereof and an expression vector including a polynucleotide encoding the light chain or
5 a fragment thereof simultaneously into a host cell.

Still further another object of the present invention is to provide a method for preparing an ENO1-specific human antibody by incubating the transformant.

The present invention also provides a composition
10 including the human antibody.

The present invention also provides a pharmaceutical composition including the human antibody as an effective ingredient.

Another object of the present invention is to provide a
15 method for treating an ENO1-overexpressed cancer, the method including administering a pharmaceutically effective amount of the human antibody to a subject with the ENO1-overexpressed cancer.

Still another object of the present invention is to
20 provide a composition including the human antibody, light or heavy chain of the human antibody or an immunologically active fragment thereof, and a radioactive isotope.

Even another object of the present invention is to provide an immunodetection method for detecting an ex vivo
25 ENO1-overexpressed cancer, including contacting the

composition for detection of the cancer with a cancer cell.

Yet another object of the present invention is to provide a method for imaging an *in vivo* ENO1-overexpressed cancer, including administering a diagnostically effective amount of
5 the composition for detection of the cancer to a subject.

Further another object of the present invention is to provide a method for treating an *in vivo* ENO1-overexpressed cancer using the composition for detection.

Still further another object of the present invention is
10 to provide a method for prognostic evaluation of a cancer treatment using the composition for detection.

【Technical Solution】

To achieve the objects, the present invention provides an ENO1-specific human antibody including a heavy chain including
15 a heavy chain variable region (V_H) including a heavy chain complementarity determining region (hereinafter, HCDR) 1 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 7 to 12, HCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 13
20 to 18, and HCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 19 to 24, or a fragment thereof; and

a light chain including a light chain variable region (V_L) including a light chain complementarity determining
25 region (hereinafter, LCDR) 1 having an amino acid sequence

selected from the group consisting of SEQ ID Nos. 31 to 36, LCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 37 to 42, and LCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID
5 Nos. 43 to 48, or a fragment thereof.

The present invention also provides a polynucleotide encoding a heavy chain of the human antibody or an immunologically active fragment thereof, and an expression vector including the polynucleotide.

10 The present invention also provides a polynucleotide encoding a light chain of the human antibody or an immunologically active fragment thereof, and an expression vector including the polynucleotide.

The present invention also provides a transformant
15 prepared by introducing an expression vector including a polynucleotide encoding a heavy chain of the human antibody or an immunologically active fragment thereof into a host cell.

The present invention also provides a transformant prepared by introducing an expression vector including a
20 polynucleotide encoding a light chain of the human antibody or an immunologically active fragment thereof into a host cell.

The present invention also provides a transformant prepared by introducing an expression vector including a polynucleotide encoding a heavy chain of the human antibody or
25 an immunologically active fragment thereof and an expression

vector including a polynucleotide encoding a light chain of the human antibody or an immunologically active fragment thereof simultaneously into a host cell.

The present invention also provides a method for
5 preparing an ENO1-specific human antibody by incubating the transformant.

The present invention also provides a composition including the human antibody.

The present invention also provides a pharmaceutical
10 composition including the human antibody.

The present invention also provides a method for treating an ENO1-overexpressed cancer, including administering a pharmaceutically effective amount of the human antibody to a subject with the cancer.

15 The present invention also provides a composition including the human antibody, a light or heavy chain of the human antibody or an immunologically active fragment thereof, and a radioactive isotope.

The present invention also provides an immunodetection
20 method for detecting an *ex vivo* ENO1-overexpressed cancer, including contacting a composition for detection of the cancer with a cancer cell.

The present invention also provides a method for imaging an *in vivo* ENO1-overexpressed cancer, the method including:

25 1) administering a diagnostically effective amount of a

composition for detection of the cancer to a subject; and

2) obtaining a detection image for the subject.

The present invention also provides a method for treating an *in vivo* ENO1-overexpressed cancer, the method including:

5 1) intravenously administering a composition including the radioactive isotope to a subject;

2) detecting the composition of Step 1) to identify tumor cells; and

3) eliminating the tumor cells identified in Step 2) by
10 surgical operation.

The present invention also provides a method for prognostic evaluation of a cancer patient, the method including:

1) intravenously administering a composition including
15 the radioactive isotope to a patient whose tumor has been eliminated;

2) detecting the composition of Step 1) to identify tumor cells; and

3) judging that all tumor cells have been eliminated when
20 tumor cells are not detected in step 2).

【Advantageous Effect】

The ENO1-specific human antibody expressed in various kinds of cancer cells of the present invention may be used in
25 diagnosis of the cancers, classification of the diseases,

visualization, treatment, and prognostic evaluation.

【Description of Drawings】

The above and other objects, features and other
5 advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a group of photos illustrating results that ENO1 genes are expressed in various cancer cell lines.

10 FIG. 2 is a group of photos illustrating results confirming that ENO1 proteins are expressed on the surface of SKOV3 (ovarian adenocarcinoma).

FIG. 3 is a group of photos illustrating results identifying induction conditions of transformant cells for
15 ENO1 expression according to IPTG concentration and processing time at 37°C and 28°C:

a: ENO1 expression according to IPTG processing time at 37°C and 28°C;

b: ENO1 expression according to IPTG concentration and
20 processing time at 37°C;

c: ENO1 expression according to IPTG concentration and processing time at 28°C; and,

d: ENO1 expression when IPTG is treated at a concentration of 0.5 mM at 37°C and 28°C for 4 hours.

25 FIG. 4 is a group of photos illustrating results of

purifying ENO1 induced at 20°C and 28°C:

a: ENO1 expression at 20°C according to IPTG concentration; and,

b: ENO1 expression at 28°C according to IPTG
5 concentration.

FIG. 5 is a graph illustrating results of phage screening in 1st to 3rd pannings.

FIG. 6 is a photo illustrating results of diversity of 6 monoclonal phage antibodies against ENO1, identified by
10 fingerprinting:

FIG. 7 is a list of sequences illustrating analysis results of polypeptides used in heavy chain and light chain CDR3 of 6 monoclonal phage antibodies against ENO1:

a: heavy chain; and
15 b: light chain.

FIG. 8 is a photo illustrating a result comparing binding specificities of commercial ENO1 antibody and phage antibody of the present invention.

FIG. 9 is a photo illustrating results, confirming that
20 the C2 phage antibody of the present invention is specifically bound to various cancer cell lines:

A: SKOV3 (ovarian adenocarcinoma);
B: Colo205 (colon adenocarcinoma);
C: 2774 (ovary serous cystadenocarcinoma);
25 a: α -ENO1 (SantaCruz) and normal mouse IgG (red color)

as a negative control group; and,

b: C2 phage antibody of the present invention and single phage as a negative control group.

FIG. 10 is a photo illustrating results, confirming that
5 ENO1-C2 and ENO1-B8 are converted into whole IgG forms.

FIG. 11 is a group of cleavage maps of pNATAB H vector and pNATAB L vector:

a: pNATAB H vector; and

b: pNATAB L vector.

10

【Best Mode】

Features and advantages of the present invention will be more clearly understood by the following detailed description of the present preferred embodiments by reference to the
15 accompanying drawings. It is first noted that terms or words used herein should be construed as meanings or concepts corresponding with the technical spirit of the present invention, based on the principle that the inventor can appropriately define the concepts of the terms to best
20 describe his own invention. Also, it should be understood that detailed descriptions of well-known functions and structures related to the present invention will be omitted so as not to unnecessarily obscure the important point of the present invention.

25 Hereinafter, the terms of the present invention will be

described.

"Variable region" means a region of an antibody molecule which specifically binds to an antigen and demonstrates modifications in sequence, which is exemplified by CDR1, CDR2, and CDR3. Between the CDRs, there is a framework region (FR) which supports the CDR loop.

"Complementarity determining region" is a loop-shaped site involved in antigen recognition, and specificity of an antibody against antigen depends on modification in that site.

"Panning" refers to a process of selecting only a phage expressing a peptide which binds to a target molecule (antibody, enzyme, cell-surface receptor, etc.) on the coat of the phage from a phage library displaying the peptide on the coat.

Hereinafter, the present invention will be described in detail.

The present invention provides an ENO1-specific human antibody including: a heavy chain including a heavy chain variable region (V_H) including a heavy chain complementarity determining region (hereinafter, HCDR) 1 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 7 to 12, HCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 13 to 18, and HCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 19 to 24, or a fragment thereof; and

a light chain including a light chain variable region (V_L) including a light chain complementarity determining region (hereinafter, LCDR) 1 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 31 to 36,
5 LCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 37 to 42, and LCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 43 to 48, or a fragment thereof.

Preferably, the heavy chain variable region has an amino
10 acid sequence selected from the group consisting of SEQ ID Nos. 25 to 30, and the light chain variable region has an amino acid sequence selected from the group consisting of SEQ ID Nos. 49 to 54.

The antibody includes not only a whole antibody, but also
15 a functional fragment of the antibody molecule. The whole antibody has a structure with two full-length light chains and two full-length heavy chains, and each light chain is linked to heavy chain by disulfide bond. The functional fragment of an antibody molecule indicates a fragment retaining a antigen-
20 binding function, and examples of the antibody fragment include (i) Fab fragment consisting of light chain variable region (V_L) , heavy chain variable region (V_H) , light chain constant region (C_L), and heavy chain 1st constant region (C_{H1}); (ii) Fd fragment consisting of V_H and C_{H1} domains; (iii) Fv
25 fragment consisting of V_L and V_H domains of a monoclonal

antibody; (iv) dAb fragment consisting of V_H domain (Ward ES *et al.*, *Nature* 341:544-546 (1989)); (v) separated CDR region; (vi) F(ab')₂ fragment including two linked Fab fragments, as a divalent fragment; (vii) single chain Fv molecule (scFv) in
5 which V_H and V_L domains are linked by a peptide linker to form an antigen binding site; (viii) bi-specific single chain Fv dimer (PCT/US92/09965), and (ix) multivalent or multi-specific diabody fragment (WO94/13804) prepared by gene fusion.

10 In the present invention, a human antibody against ENO1 was obtained as scFV by using phage display technology and screened as a mono phage clone. As a result, 6 kinds of ENO1-specific monoclonal phages were obtained.

In a specific example of the present invention, because
15 ENO1 whose gene is confirmed to be expressed in various cancer cell lines the activity (see FIG. 1) is usually expressed on the surface of cancer cell lines (see FIG. 2), it is available as a target protein of various cancers. The ENO1 expressed and purified through recombinant technology (see FIGs. 3 and
20 4) was reacted with a library phage constructed from human naive scFV library cells having diversity, followed by panning and screening of monoclones strongly binding to the ENO1 antigen (see Tables 3 & 4 and FIG. 5). The selected monoclones were identified by fingerprinting (see FIG. 6),
25 followed by sequencing to identify CDR regions of V_H and V_L of

the antibody (see SEQ ID Nos. 7 to 54 in Table 7 and FIG. 7). The Ig BLAST program of NCBI (<http://www.ncbi.nlm.nih.gov/igblast/>) was used for identification of similarity between the antibody and a germ line antibody group (see Table 8). The heavy chain exhibited a homology of 84% to 96% with the sequence of human germ cell line while the light chain exhibited a homology of 86% to 96%. 4 kinds of VH5-51 and each kind of VH3-9 and VH3-30 were included as a heavy chain, while 5 kinds of lambda and one kind of kappa chain were included as a light chain. Analysis showed that polypeptides of each of human heavy and light chains were different, respectively. It was confirmed that the selected monoclonal antibodies could detect antigen proteins clearly at a similar level to that of a commercially available non-human antibodies (see FIG. 8), and that the antibodies were specifically bound to ENO1 in various cancer cells (see FIG. 9).

The present invention also provides a polynucleotide encoding a heavy chain of the human antibody or an immunologically active fragment thereof, and an expression vector including the polynucleotide.

The present invention also provides a polynucleotide encoding a light chain of the human antibody or an immunologically active fragment thereof, and an expression vector including the polynucleotide.

In a specific embodiment of the present invention, ENO1 expressed on the surface of various cancer cell lines (see FIG. 1 and 2), and expressed and purified through recombinant technology was reacted with a library phage constructed from human naive scFV library cells having diversity, followed by panning and screening of monoclones strongly binding to the ENO1 antigen (see Tables 3 & 4 and FIG. 5). The selected monoclones were identified by fingerprinting (see FIG. 6), followed by sequencing to identify CDR regions of V_H and V_L of the antibody (see SEQ ID Nos. 7 to 54 in Table 7 and FIG. 7). 4 kinds of VH5-51 and each kind of VH3-9 and VH3-30 were included as a heavy chain, while 5 kinds of lambda and one kind of kappa chain were included as a light chain. Analysis showed that polypeptides of each of human heavy and light chains were different, respectively. It was confirmed that the selected monoclonal antibodies could detect antigen proteins clearly at a similar level to that of a commercially available non-human antibodies (see FIG. 8), and that the antibodies were specifically bound to ENO1 in various cancer cells (see FIG. 9).

In the polynucleotide encoding a light and heavy chain of the human antibody of the present invention or a fragment thereof, due to degeneracy of the codon or in consideration of a preferred codon in an organism where light and heavy chains of the human antibody or a fragment thereof are to be

expressed, various modifications may be made in a coding region within a scope that the amino acid sequences of light and heavy chains or a fragment thereof are not changed, and various changes or modifications may be made even in portions
5 other than the coding region within a scope that the gene expression is not affected. It will be appreciated by those skilled in the art that these modified genes are also included within the scope of the present invention. That is, one or more nucleotides may be modified by substitution, deletion,
10 insertion, or a combination thereof as long as the polynucleotide of the present invention encodes a protein with an equivalent activity thereof, and they are also included in the present invention. The sequence of the polynucleotide may be a single or double chain, and a DNA or RNA (mRNA) molecule.

15 In preparation of the expression vector, an expression control sequence such as a promoter, a terminator, an enhancer, etc., a sequence for membrane targeting or secretion, etc. may be appropriately selected according to a kind of host cell in which light and heavy chains of the human
20 antibody or a fragment thereof are to be produced, and may be variously combined according to its purpose.

The expression vector of the present invention includes, but is not limited to, a plasmid vector, a cosmid vector, a bacteriophage, and a viral vector. A suitable expression
25 vector may include expression regulatory elements such as a

promoter, an operator, an initiation codon, a stop codon, a polyadenylation signal, and an enhancer and a signal sequence or leader sequence for membrane targeting or secretion, and may be variously prepared according to its purpose. A
5 promoter of the expression vector may be constitutive or inductive. Examples of the signal sequence for use may include, but is not limited to, a PhoA signal sequence and an OmpA signal sequence for genus *Escherichia* hosts; an α -amylase signal sequence and a subtilisin signal sequence for genus
10 *Bacillus* hosts; an MF α signal sequence and an SUC2 signal sequence for yeast hosts; and an insulin signal sequence, an α -interferon signal sequence, and an antibody molecule signal sequence for animal cell hosts. In addition, the expression vector may include a selection marker for selecting host cells
15 containing the vector, and a replication origin when it is a replicable expression vector.

The present invention also provides a transformant prepared by introducing an expression vector including a polynucleotide encoding a heavy chain of the human antibody or
20 an immunologically active fragment thereof into a host cell.

The present invention also provides a transformant prepared by introducing an expression vector including a polynucleotide encoding a light chain of the human antibody or an immunologically active fragment thereof into a host cell.

25 The present invention also provides a transformant

prepared by introducing an expression vector including a polynucleotide encoding a heavy chain of the human antibody or a fragment thereof and an expression vector including a polynucleotide encoding a light chain of the human antibody or
5 a fragment thereof simultaneously into a host cell.

In a specific example of the present invention, genes encoding light and heavy chains of a monoclonal phage were obtained and linked to a vector, respectively, and then the expression vectors were simultaneously introduced into a host
10 cell to prepare a whole human IgG antibody for *in vivo* diagnosis and treatment of cancers.

The expression vector according to the present invention may be transformed into a suitable host cell, for example, *E. coli* or yeast cell, and the transformed host cell may be
15 incubated to produce light and heavy chains of the human antibody of the present invention or a fragment thereof in mass quantities. Incubation methods and media conditions suitable for a kind of host cell may be easily chosen from those known to those skilled in the art. The host cell may be
20 a prokaryote such as *E. coli* or *Bacillus subtilis*. In addition, it may be a eukaryotic cell derived from a yeast such as *Saccharomyces cerevisiae*, an insect cell, a vegetable cell, and an animal cell. More preferably, the animal cell may be an autologous or allogeneic animal cell. A
25 transformant prepared through introduction into an autologous

or allogeneic animal cell may be administered to a subject for use in cellular therapy for cancer. As for a method for introducing an expression vector into the host cell, it is possible to use any method if it is known to those skilled in the art.

The present invention also provides a method for preparing an ENO1-specific human antibody by incubating the transformant.

Specifically, the present invention provides a method for preparing an ENO1-specific human antibody, the method including:

- 1) incubating the transformant; and,
- 2) purifying the human antibody from the medium.

As for the culture medium, it is desirable to select and use a culture medium suitable for the transformant among those known to those skilled in the art. As for the method for purifying human antibodies, it is possible to use any method known to those skilled in the art.

The present invention also provides a composition including the human antibody.

The present invention also provides a pharmaceutical composition including the human antibody.

The pharmaceutical composition may be useful for prevention and treatment of an ENO1-overexpressed cancer. The ENO1-overexpressed cancer is preferably one selected from the

group consisting of, but not limited to, lung cancer, colorectal cancer, breast cancer, ovarian cancer, renal cell cancer, and prostate cancer, and includes all the ENO1-overexpressed cancers.

5 The pharmaceutical composition of the present invention may selectively contain the ENO1-specific human antibody or the transformant, and may additionally contain one or more effective ingredients exhibiting functions identical or similar to those of the ingredient. For administration, the
10 pharmaceutical composition of the present invention may be formulated by including one or more pharmaceutically acceptable carriers in addition to the effective ingredients described above. For example, the pharmaceutically acceptable carrier includes saline solution, sterilized water, Ringer's
15 solution, buffered saline solution, dextrose solution, maltodextrin solution, glycerol, ethanol, liposome, and at least one combination thereof, and other general additives such as antioxidants, buffer solution, bacteriostatic agents, etc. may be added if necessary. In addition, it may be
20 formulated in the form of an injectable formulation such as aqueous solution, suspension, emulsion, etc. by additionally adding diluent, dispersing agent, surfactant, binder and lubricant, and antibodies and other ligands specific to a target cell may be used in combination with the carrier to be
25 specifically reacted with the target cell. Furthermore, the

composition may be preferably formulated according to each disease or ingredient using a suitable method in the art or a method which is taught in Remington's Pharmaceutical Science, Mack Publishing Company, Easton PA.

5 The pharmaceutical composition of the present invention may be parenterally administered, and the parenteral administration is effected by subcutaneous injection, intravenous injection, intramuscular injection, or intrapleural injection. For parenteral administration, the
10 pharmaceutical composition of the present invention may be mixed with a stabilizer or buffer to prepare a solution or suspension, which may then be provided as ampoules or vials each containing a unit dosage form.

 The pharmaceutical composition of the present invention
15 may be prepared in various forms according to the route of administration. For example, the pharmaceutical composition of the present invention may be formulated to a sterilized aqueous solution or dispersion for injection, or may be prepared in a freeze-dried form through a freeze-drying
20 technique. The freeze-dried pharmaceutical composition may be stored typically at about 4°C and may be reconstituted with a stabilizer that may contain an adjuvant such as saline solution and/or HEPE.

 In a method of the present invention, factors affecting
25 the amount of the pharmaceutical composition to be

administered include, but are not limited to, administration mode, administration frequency, specific disease under treatment, severity of disease, history of disease, whether the subject is under treatment in combination with other
5 therapeutics, the subject's age, height, weight, health, and physical conditions. As the patient's weight under treatment increases, the pharmaceutical composition of the present invention may preferably be administered in increasing amounts.

10 The present invention also provides a method for treating an ENO1-overexpressed cancer, the method including administering a pharmaceutically active amount of the human antibody to a subject with the cancer.

The subject applicable in the present invention is a
15 vertebrate, preferably a mammal, more preferably an experimental animal such as mouse, rabbit, guinea pig, hamster, dog, and cat, and most preferably a primate such as chimpanzee and gorilla.

The method for administering the human antibody of the
20 present invention may be conducted by parenteral administration (for example, intravenous, subcutaneous, intraperitoneal, or local administration) according to the purpose of use, and preferably by intravenous administration. In administration for solid cancer, local administration may
25 be often preferable for rapid and facilitated access of the

antibody. The dose may vary depending on weight, age, sex, and health condition of a patient, diet, administration time, administration method, excretion rate, and severity of disease. The single dose is in the range of 5 to 500 mg/m²,
5 which may be administered daily or weekly. The effective amount may be controlled at the discretion of a doctor treating the patient.

The human antibody of the present invention may be used alone or in combination with surgery, hormone therapy,
10 chemical therapy, and a biological response controller for treatment of a patient.

The present invention also provides a composition including the human antibody, light and heavy chains of the human antibody, or an immunologically active fragment thereof,
15 and a radioactive isotope.

The composition may be useful for radioimmuno treatment and detection of an ENO1-overexpressed cancer.

Examples of preferred radioactive isotopes include ³H, ¹¹C, ¹⁴C, ¹⁸F, ⁶⁴Cu, ⁷⁶Br, ⁸⁶Y, ^{99m}Tc, ¹¹¹In, ¹²³I, ¹⁷⁷Lu, and a
20 mixture or combination thereof. The radioactive isotope is characterized by the fact that it is bound to a human antibody and included in a carrier to which the human antibody is bound.

The present invention also provides an immunodetection
25 method for detecting an ex vivo ENO1-overexpressed cancer, the

method including: contacting a composition including the radioactive isotope with cancer cells.

The composition for detection may be bound to a solid substrate in order to facilitate the subsequent steps such as washing or separation of complexes. The solid substrate includes, for example, synthetic resin, nitrocellulose, glass substrate, metal substrate, glass fiber, microsphere, microbead, etc. The synthetic resin includes polyester, polyvinyl chloride, polystyrene, polypropylene, PVDF, nylon, etc.

In addition, cancer cell may be appropriately diluted before it is contacted with the composition for detection.

The present invention also provides a method for imaging an ENO1-overexpressed cancer, the method including 1) administering a diagnostically effective amount of a compound including the radioactive isotope to a subject; and

2) obtaining a detection image for the subject.

The detection image is characterized by the fact that it is obtained by near-infrared light imaging, PET, MRI, or ultrasonic imaging.

The present invention also provides a method for treating an *in vivo* ENO1-overexpressed cancer, the method including:

- 1) intravenously administering a composition including the radioactive isotope to a subject;
- 2) detecting the composition of Step 1) to identify tumor

cells; and

3) eliminating the tumor cells identified in Step 2) by surgical operation.

Furthermore, the present invention provides a method for prognostic evaluation of a cancer patient, the method including:

1) intravenously administering a composition including the radioactive isotope to a patient whose tumor has been eliminated;

2) detecting the composition of Step 1) to identify tumor cells; and

3) judging that all tumor cells have been eliminated when tumor cells are not detected in step 2).

15 **【Mode for Invention】**

Hereinafter, the present invention will be described in more detail with reference to examples.

However, the following examples are provided for illustrative purposes only, and the scope of the present invention should not be limited thereto in any manner.

<Example 1> Identification of ENO1 expression in various cancer cell lines through RT-PCR

QiagenEasyRNA kit (USA) was used in 15 kinds of different cancer cell lines in Table 1 to extract a total RNA, and each of 1 μ g of Superscriptase II Reverse-Transcriptase

(Invitrogen, USA) as a total RNA was used respectively to synthesize a cDNA. The cDNA was diluted with sterilized distilled water to prepare a 2% cDNA, and then each of 10 pmole/ μ l of Taq DNA polymerase (Solgent, Korea), forward primer (SEQ ID No. 1: 5'-GAATTGAAGAGGAGCTGGGC-3'), and reverse primer (SEQ ID No. 2: 5'-GTGTAGCCAACAGGTGACCG-3') was used to perform a PCR under conditions described in Table 2. GAPDH was used as gel loading control group.

10 Table 1

Cancer	Cell line	Manufacturer	Cancer	Cell line	Manufacturer
Lung	NCI-H226	ATCC	Colorectal	WiDr	ATCC
Lung	NCI-H322	ATCC	Ovarian	SKOV3	ATCC
Lung	NCI-H460	ATCC	Colorectal	HCT-15	ATCC
Colorectal	A549	ATCC	Ovarian	MDAH-2774	ATCC
Lung	HOP-92	NCI/NIH	Renal cell	UO31	NCI
Breast	MDA-MB435	ATCC	Breast	MCF-7	ATCC
Colorectal	Colo205	ATCC	Prostate	DU145	ATCC
Colorectal	SW480	ATCC			

Table2

Temperature	Time	Cycle
95°C	3 min	30
95°C	30 sec	
55°C	30 sec	
72°C	1 min	
72°C	10 min	

As a result, as shown in FIG. 1, it was confirmed that ENO1 genes were expressed in various cancer cell lines. That is, ENO1 may be used as a target protein for various cancers.

<Example 2> Investigation of ENO1 expression location using immunofluorescence microscopy

1× PBS was used to prepare all the solutions. Coverglass was placed in a 6-well plate, and coating with 20% FBS was performed. Subsequently, SKOV3 ovarian cancer cell line with dominant ENO1 expression was incubated in the plate. After an incubation for 24 hours, the cell line was immobilized with 4% paraformaldehyde for 10 minutes, and membrane permeabilization was carried out with 0.05% Triton X-100 for 5 minutes. The cell line was blocked with 3% normal goat serum (vector Lab. Inc, USA), and then ENO1 antibodies diluted at 1:50 in a dilution buffer (0.1% BSA, 0.05% Triton X-100) was used to stain the cell line for 1.5 hours, followed by washing three times with 1× PBS. The secondary antibody (anti-mouse IgG-FITC, Vector Lab. Inc. FI-200, USA) diluted at 1:2500 in the dilution buffer was used to stain the cell line for 45 minutes, followed by washing three times with 1× PBS. The cell line was stained with DAPI (Glass-Slide) for 5 minutes and washed with PBS. The coverglass was transferred and mounted onto a Glass-Slide, and nail-polish was used for sealing. The reaction results were analyzed by a fluorescent microscope (MFI-4000, Leica).

As a result, as shown in FIG. 2, it was confirmed that ENO1 was present in cytoplasm and cell membrane of SKOV3 cell line and expressed generally on the surface of cells, which is

identical to results confirmed in previous reports (Chang GC et al., *Clin Cancer Res* 2006; 12:574654; Satoshi Ito et al., *Cancer Sci.* 2007, 98 (4): 499-505).

<Example 3> Preparation of ENO1 antigen protein

5 <3-1> ENO1 gene cloning

A plasmid (IRAT-14-B09) containing a human ENO1 gene was provided from KUGI (Korean UniGene Information) of the Center for Functional Analysis of Human Genome in Korea Research Institute of Bioscience and Biotechnology. The plasmid was
10 used as a template DNA. A forward primer (SEQ ID No. 3: CGCGGATCCTCTATTCTCAAGATCCATGC) and a reverse primer (SEQ ID No. 4: CCGCTCGAGCTTGGCCAAGGGGTTTCTGAA) were used to amplify the gene under the following conditions, followed by subcloning into a pET28A (Novagen 69864-3, USA). PCR
15 conditions are as follows: when a total reaction reagent was 50 μ l, 100 ng of the template was introduced and a reaction at 94°C for 2 minutes, 30 cycles of reactions at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for a half minutes, and at a reaction at 72°C for 10 minutes were
20 performed to get a PCR product. Furthermore, the base sequence of the subcloned vector was identified

<3-2> Expression and purification of ENO1 protein

The subcloned vector was transformed with BL21 (DE3), and
25 then inducing conditions for protein expression were examined.

The vector was treated with IPTG at concentrations of 0.1, 0.5, and 1 mM at 37°C and 28°C and cells were collected at 1, 2, and 4 hours. Followed by adjusting the final O.D values, subsequently, the protein expression aspect in whole cell
5 lysate was identified by SDS-PAGE.

As a result, as shown in FIG. 3, the expression was the most dominant when 0.5 mM of IPTG was treated at 28°C for 4 hours. However, the expression was quite favorable at 37°C without much fluctuation over according to time. In addition,
10 a lot of insoluble proteins were observed, but soluble proteins were also expressed to some degree.

Because protein is stable at low temperatures, inducing conditions for protein expression were compared at decreased temperatures, 28°C and 20°C. Then, 0.5 mM IPTG was treated for
15 4 hours to incubate each of 300 ml of protein. A Ni-NTA column (Qiagen, USA) was used for the protein to be eluted and purified with 500 mM Imidazole, and then the purity of ENO1 purified was identified by SDS-PAGE according to concentrations compared to BSA.

20 As a result, as shown in FIG. 4, about 500 µg and 1 mg of the purified ENO1 were obtained at 28°C and 20°C, respectively, and used in panning for antibody preparation.

<Example 4> Construction of library phage

2.7 x 10¹⁰ human naive scFv library cells having
25 diversity were incubated in a medium (3 L) containing 2×YTCM

[17 g of Tryptone (CONDA, 1612.00), 10 g of yeast extract (CONDA, 1702.00), 5 g of NaCl (Sigma, S7653-5 kg), 34 $\mu\text{g}/\text{ml}$ of chloramphenicol (Sigma, C0857)], 2% glucose (Sigma, G5400), and 5 mM MgCl_2 (Sigma, M2393) at 37°C for 2-3 hours
5 (OD₆₀₀=0.5~0.7). Then, the cells were infected with helper phage, followed by incubation in a medium containing 2xYTCMK [2xYT CM, 70 $\mu\text{g}/\text{ml}$ of Kanamycin (Sigma, K1876), 1 mM IPTG (ELPISBIO, IPTG025)] at 30°C for 16 hours. The incubated cells were centrifuged (4500 rpm, 15 min, 4°C) to obtain a
10 supernatant. The supernatant was treated with PEG (Fluka, 81253) and NaCl (Sigma, S7653) until the two reagents became 4% and 3%, respectively. The reactant was centrifuged again (8000 rpm, 20 min, 4°C). The pellet was dissolved in PBS, which proceeded to centrifugation again (12000 rpm, 10 min,
15 4°C). As a result, the supernatant containing library phage was obtained, which was transferred to a new tube and stored at 4°C.

<Example 5> Preparation of monoclonal antibody

20 <5-1> Panning process

An immunosorb tube (Nunc 470319) was coated with 30 μg of the purified ENO1-antigens (TMPS4-FLAG and TM-EK) obtained in Example 3 using 4 ml of a coating buffer [1.59 g of Na_2CO_3 (Sigma, S7795), 2.93 g of NaHCO_3 (Sigma, S8875), 0.2 g of NaN_3
25 (Sigma, S2002)] at 4°C for 16 hours with rotator. Then, the

antigen was dissolved in PBS at room temperature for 2 hours, followed by blocking in the immunotube using skim milk [(BD,232100)-4% in 1XPBS]. 2 ml of library phage constructed in Example 4 was added into the immunotube, followed by
 5 reaction at room temperature for 2 hours. The immunotube was washed five times with PBST (0.05%) and twice with PBS. After washing, antigen specific scFV-phage was eluted using 100 mM TEA (Sigma T-0886). *E. coli* (XL1-blue, stratagene, 200249) was transfected with the eluted phage, followed by
 10 amplification. The 2nd and 3rd pannings was performed on the phage amplified at the first panning by the same manner as described above except that washing times with PBST were increased (2nd: 13 times, 3rd: 23 times).

As a result, as shown in Table 3, it was confirmed that
 15 colony titer of the phage against the antigen was increased at least 1000 times in the 3rd panning.

【Table 1】

Target antigen	Panning	Initial phage number	Binding phage number
ENO1	1 st	5×10^{12}	1.2×10^7
	2 nd	1.2×10^{13}	3×10^6
	3 rd	2.6×10^{13}	2.5×10^9

<5-2> Screening of phage antibody by phage ELISA

<5-2-1> Identification of panning results

Cell stocks obtained from the 1st-3rd pannings and stored as frozen were dissolved in a medium containing 5 ml of 2×YTCM, 2% glucose, and 5 mM MgCl₂ to make OD₆₀₀ as 0.1. Then, the cells were incubated at 37°C for 2-3 hours (OD₆₀₀=0.5~0.7), which were infected with M1 helper phage. Then, the cells were incubated in a medium containing 2×YTCMK, 5 mM MgCl₂ and 1 mM IPTG at 30°C for 16 hours. At this point, a single phage antibody, which was specifically binding to WW45 antigen associated with a developmental process unrelated to VEGF and constructed by a method in Example of the present invention, was used as a control group. The incubated cells were centrifuged (4500 rpm, 15 min, 4°C), and the supernatant was transferred to a new tube (1st ~ 3rd panning poly scFv-phage). A 96-well immuno-plate (NUNC 439454) was coated with ENO antibody-His and 6xHis antigens (100 ng/well) using a coating buffer at 4°C for 16 hours, followed by blocking with skim milk dissolved in PBS (4%). Each well of the 96-well immuno-plate was washed with 0.2 ml of PBS-tween20 (0.05%). 100 µl of the 1st - 3rd panning poly scFV-phage was added into each well, followed by reaction at room temperature for 2 hours. Again, each well was washed four times with 0.2 ml of PBS-tween20 (0.05%). The secondary antibody anti-M13-HRP (Amersham 27-9421-01) was diluted at 1:2000, followed by

reaction at room temperature for 1 hour. The reactant was washed with 0.2 ml of PBS-tween20 (0.05%). An OPD tablet (Sigma 8787-TAB) was added into a PC buffer [5.1 g of $C_6H_8O_7H_2O$ (Sigma, C0706), 7.3 g of Na_2HPO_4 (Sigma, S7907)] to make a substrate solution, which was added into each well by 100 μ l/well, followed by color development for 10 minutes. The optical density was measured at 490 nm by using a spectrophotometer (MolecularDevice, USA).

As a result, as shown in Figure 5, it was confirmed that binding capacities to ENO1 antigen were increased from the 2nd panning to reach a saturated state, and binding capacities to the antigen were enhanced in the 3rd panning.

<5-2-2> Selection of monoclonal antibodies

Colonies obtained from a polyclonal antibody groups (the 2nd and 3rd panning) having strong binding capacities were incubated in a 96-deep well plate (Bioneer, 90030) containing 1 ml of a medium supplemented with 2 $\times$ YTCM, 2% glucose and 5 mM $MgCl_2$ at 37°C for 16 hours. 100-200 μ l of the solution was incubated in 1 ml of a medium supplemented with 2 $\times$ YTCM, 5 mM $MgCl_2$, and 1 mM IPTG, which was loaded in a 96-deep well plate at 37°C for 2-3 hours, followed by inoculation at an initial OD_{600} value of 0.1. The cells were infected with M1 helper phage (MOI=1:20) and the infected cells were cultured in a medium supplemented with 2 $\times$ YTCMK, 5 mM $MgCl_2$, and 1 mM IPTG at

30°C for 16 hours. The cultured cells were centrifuged (4500 rpm, 15 min, 4°C) and a supernatant was obtained, to which 4% PEG 6000 and 3% NaCl were added. Upon completion of dissolving, reaction was induced in ice for 1 hour. The
5 reactant was centrifuged (8000 rpm, 20 min, 4°C) and pellet was dissolved in PBS. Centrifugation (12000 rpm, 10 min, 4°C) was performed again and a supernatant was obtained, from which the 3rd panning monoclonal scFv phage was obtained. The phage was transferred to a new tube and stored at 4°C.

10 A 96-well immuno-plate was coated with the two antigens (100 ng/well) at 4°C for 16 hours, followed by blocking with skim milk dissolved in PBS (4%). Each well of the 96-well immuno-plate was washed with 0.2 ml of PBS-tween20 (0.05%). 100 µl of the 3rd panning monoclonal scFV-phage was added to
15 each well, followed by reaction at room temperature for 2 hours. Each well was washed four times with 0.2 ml of PBS-tween20 (0.05%). The secondary antibody anti-M13-HRP was diluted at 1:2000, followed by reaction at room temperature for 1 hour. The plate was washed with 0.2 ml of PBS-tween20
20 (0.05%), followed by color development. The optical density was measured at 490 nm.

As a result, as shown in Table 4, ELISA was performed on monoclones selected from the 2nd and 3rd polyphage pools to select 40 monoclonal phages having ENO1 binding capacity
25 values of 1.5 or more (highlighted in Table 4).

【Table 4】

Eno ≥ 1.5	Eno	anti-myc	His		Eno	anti-myc	His		Eno	anti-myc	His
A1	0.271	0.240	0.308	A5	2.649	0.342	0.477	A9	2.129	0.190	0.139
B1	0.159	0.086	0.136	B5	1.915	2.065	1.725	B9	2.849	0.925	0.598
C1	0.322	0.182	0.304	C5	1.370	1.684	1.307	C9	1.912	0.239	0.187
D1	2.823	0.291	0.269	D5	2.574	0.312	0.321	D9	2.345	0.231	0.244
E1	0.160	0.109	0.182	E5	0.309	0.254	0.267	E9	0.761	0.079	0.115
F1	2.682	0.232	0.225	F5	0.123	0.055	0.156	F9	1.383	0.803	0.603
G1	2.816	0.228	0.267	G5	1.903	0.438	0.442	G9	1.585	0.118	0.139
H1	0.674	0.224	0.176	H5	0.711	0.398	0.646	H9	1.345	0.130	0.147
A2	0.409	0.234	0.359	A6	0.144	0.228	0.191	A10	1.949	0.214	0.241
B2	2.753	0.331	0.229	B6	0.072	0.049	0.156	B10	0.073	0.047	0.162
C2	3.036	1.525	1.071	C6	2.343	0.146	0.160	C10	1.771	0.115	0.132
D2	0.693	0.777	0.682	D6	0.564	0.595	1.016	D10	2.018	0.169	0.186
E2	0.206	0.070	0.261	E6	2.529	0.311	0.230	E10	1.429	0.102	0.136
F2	2.944	1.422	1.068	F6	2.746	0.787	0.698	F10	1.894	0.157	0.163
G2	0.178	0.164	0.171	G6	0.064	0.050	0.187	G10	2.243	0.214	0.216
H2	2.122	1.356	1.289	H6	0.843	0.957	0.651	H10	1.951	0.161	0.204
A3	1.841	1.099	0.830	A7	1.476	0.120	0.116	A11	1.809	0.171	0.184
B3	1.678	0.549	0.495	B7	1.461	0.114	0.137	B11	1.641	0.145	0.190
C3	0.229	0.245	0.321	C7	1.008	0.064	0.082	C11	0.191	0.111	0.169
D3	2.699	0.294	0.219	D7	2.726	0.578	0.408	D11	1.736	0.101	0.118
E3	1.205	0.350	0.346	E7	1.366	0.095	0.143	E11	1.099	0.199	0.193
F3	0.554	0.616	0.525	F7	1.675	0.119	0.130	F11	1.721	0.128	0.118
G3	0.101	0.070	0.182	G7	0.627	0.400	0.456	G11	0.213	0.084	0.120
H3	0.092	0.058	0.172	H7	0.071	0.048	0.058	H11	1.312	0.078	0.102
A4	2.492	0.145	0.160	A8	1.256	0.063	0.089	A12	0.739	0.088	0.097
B4	1.106	0.703	0.620	B8	2.672	0.279	0.215	B12	1.689	0.132	0.123
C4	1.808	0.702	0.607	C8	1.486	0.157	0.249	C12	1.349	0.108	0.126
D4	2.707	0.341	0.343	D8	1.441	0.123	0.164	D12	1.476	0.136	0.145
E4	2.767	0.304	0.268	E8	1.052	0.076	0.115	E12	1.618	0.171	0.159
F4	0.194	0.170	0.390	F8	0.638	0.058	0.069	F12	2.067	1.124	1.105
G4	2.430	0.144	0.135	G8	0.681	0.076	0.112	G12	1.102	0.814	0.905
H4	0.898	2.424	0.917	H8	0.825	0.175	0.167	H12	0.173	0.051	0.130

<5-3> Identification of monoclonal phages and examination thereof

<5-3-1> Verification by fingerprinting

1 μl of the fifty monoclonal cells firstly selected, 0.2
 5 μl of Taq DNA polymerase (Gendocs, Korea) (5 U/ μl), 0.2 μl of
 each forward primer (pelB5, SEQ. ID. No. 5: 5'-
 CTAGATAACGAGGGCAAATCATG-3') and reverse primer (cla3, SEQ. ID.
 No. 6: 5'-CGTCACCAATGAAACCATC-3') at 50 p/ μl , 3 μl of 10 $\times$
 10 buffer, 0.6 μl of 10 mM dNTP mix, and 24.8 μl of distilled
 water were mixed to perform a colony PCR (iCycler iQ, BIO-
 RAD). PCR conditions are as shown in Table 5.

【Table 5】

Temperature	Time	Cycle
95°C	5 min	
95°C	30 sec	30
56°C	30 sec	
72°C	1 min	
72°C	10 min	
4°C		

The colony PCR product was identified on a 1% agarose gel
 15 (Seakem LE, CAMERES 50004). 0.2 μl of *Bst*NI
 (Roche11288075001, 10 U/ μl) was added to perform a reaction at
 37°C for 2-3 hours. Reaction conditions are as shown in Table
 6. The fragmented product was identified on an 8% DNA

polyacrylamide gel.

【Table 6】

10X Buffer	3 μl
colony PCR product	10 μl
BstNI (10U/ μl)	0.2 μl
Distilled water	16.8 μl

As a result, as shown in Figure 6, fragments of
5 monoclonal phage antibodies digested by *Bst*NI were proved to
have diversity.

<5-3-2> Verification by base sequence analysis

6 kinds of the monoclonal phages were incubated in a
10 medium (5 ml) supplemented with 2×YTCM, 2% glucose, and 5 mM
MgCl₂ at 37°C for 16 hours. A DNA purification kit (Nuclogen
5112) was used for the incubated monoclonal phages to obtain a DNA,
and then sequencing was performed by using a pelB5 primer of
SEQ ID No. 125 (Solgent, Korea).

15 As a result, as shown in Table 7 and FIG. 7, CDR regions
of V_H and V_L of the selected antibody were identified.

Similarity between the antibody and germ line antibody
group was investigated by Ig BLAST program of NCBI
(//www.ncbi.nlm.nih.gov/igblast/). As a result, 6 kinds of
20 ENO1 specific phage antibodies were obtained, and the result
was summarized and presented in Table 8. In particular, The

heavy chain exhibited a homology of 84% to 96% with the sequence of human germ cell line while the light chain exhibited a homology of 86% to 96%. 4 kinds of VH5-51 and each kind of VH3-9 and VH3-30 were included as a heavy chain, while 5 kinds of lambda and one kind of kappa chain were included as a light chain. Analysis showed that polypeptides of each of human heavy and light chains were different, respectively.

10 【Table 7】

Clone Name	Heavy Chain			Light Chain		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
A3	NHWIG : SEQ ID No. 7	IIHPRDS DTRY SPAFQG : SEQ ID No. 13	HKVSGNSWY SGSLAY : SEQ ID No. 19	SGDALSKQY AS : SEQ ID No. 31	KDTERPL : SEQ ID No. 37	QSITDKSGTD VI : SEQ ID No. 43
B3	SYWLT : SEQ ID No. 8	RIDPNSDFS LY NPAFQG : SEQ ID No. 14	QHNGNGDLL NALDI : SEQ ID No. 20	SGDVLSKQY AY : SEQ ID No. 32	KDNVRPS : SEQ ID No. 38	QSGDRSGSYR VL : SEQ ID No. 44
C2	SNWIS : SEQ ID No. 9	AIDPLDSYTN YSPSFQG : SEQ ID No. 15	SNQATFDV : SEQ ID No. 21	SGTSSNIGNN FVY : SEQ ID No. 33	RNDQRPS : SEQ ID No. 39	ATWDDSLSG YV : SEQ ID No. 45
C4	EYAMH : SEQ ID No. 10	HISWNSGSIA YGDSVKG : SEQ ID No. 16	GSGSGIYYKN YGMDV : SEQ ID No. 22	SGDNLRTKY VS : SEQ ID No. 34	QDTRRPS : SEQ ID No. 40	MTWDVDTTT MI : SEQ ID No. 46
B8	THWIG : SEQ ID No. 11	IIHPGNSDIRY SPSFQG : SEQ ID No. 17	NSDIGTNR RM GFDI : SEQ ID No. 23	SGDALSKKEYS Y : SEQ ID No. 35	KDSERPS : SEQ ID No. 41	QSVDSSTSV V : SEQ ID No. 47
F12	NYDMH : SEQ ID No. 12	LIAYDGSYQH HADSVQA : SEQ ID No. 18	WNWGLGFDF : SEQ ID No. 24	RASQNIANS L H : SEQ ID No. 36	YASQSI S : SEQ ID No. 42	MQGSSRPRI : SEQ ID No. 48

【Table 8】

Clone Name	VH			VL			Group
	Families	Identities	CDR3-a.a seq	Families	Identities	CDR3-a.a seq	
A3	VH5-51	96.3% (284/295)	HKVSGNSWYSGS LAY: SEQ ID No. 19	V2-17	91.4% (266/291)	QSTIDKSGTDV I: SEQ ID No. 43	1
B3	VH5-51	84.1% (248/295)	QHNGNGDLLNAL DI: SEQ ID No. 20	V2-17	94.5% (273/289)	QSGDRSGSYR VL: SEQ ID No. 44	2
C2	VH5-51	89.4% (262/293)	SNQATFDV: SEQ ID No. 21	V1-17	94.1% (270/287)	ATWDDSLSGY V: SEQ ID No. 45	3
C4	VH3-9	94.9% (277/292)	GSGSGIYYKNYG MDV: SEQ ID No. 22	V2-1	86.5% (244/282)	MTWDVDITS MI: SEQ ID No. 46	4
B8	VH5-51	91.8% (269/293)	NSDIGTNRRMGFD I: SEQ ID No. 23	V2-17	95.8% (274/286)	QSVDSSTSVV : SEQ ID No. 47	5
F12	VH3-30	89.3% (259/290)	WNWGLGFDF: SEQ ID No. 24	A26	90.6% (250/276)	MQGSSRPRI: SEQ ID No. 48	6

<Example 6> Analysis of characteristics of human antibody against ENO1

5 <6-1> Western blot analysis of phage

Two sheets of 10% SDS-PAGE gel into which the antigen ENO1 is loaded (200 and 400 ng/well) were electrophoresized at 100 V for 2 hours and transferred to NC membrane (Millipore Cat. No. HATF00010) at 85 V for 2 hours, followed by blocking
10 with skim milk dissolved in TBST (4%) at 4°C overnight. Subsequently, α-ENO1 antibody (1 mg/ml; SC-100812) prepared from a commercially available mouse was diluted at 1:100 in skim milk dissolved in TBST. A supernatant of the monoclonal C2 phage antibody selected in Example 5 was diluted at 1:50 in
15 skim milk dissolved in TBST, followed by reaction at room temperature for 1 and a half hours. The dilution was washed

five times with TBST, each of anti-mouse IgG-HRP (Sigma) and anti-M13-HRP (Amersham bioscience) was used for dilution at 1:1000 in skim milk in TBST (4%), followed by reaction at room temperature for 30 minutes. Then, it was washed by the same manner. After washing, developments were performed (Intron, Cat. No. 12145) to compare amounts of antigen proteins which could be detected by a commercially available antibody and a monoclonal phage antibody.

As a result, as shown in FIG. 8, C2 phage antibodies of the present invention was used to detect about 48 KDa of antigen protein clearly at a similar level to that of a commercially available antibody.

<6-2> Phage FACS analysis

Ovarian cancer cell line (SKOV3, 2774; ATCC) and colorectal cancer cell line were washed twice with PBS in a 100 mm plate. An enzyme-free PBS-based lysis buffer (Gibco) was added into the plate, followed by incubation at 37°C for 10 minutes. Subsequently, cells were collected by a scraper and centrifuged at 1300 rpm for 3 minutes. The pellet was washed twice with a 2% PBF solution (1×PBS supplemented with 2% FBS), followed by resuspension with 2% PBF solution at a concentration of $\geq 5 \times 10^5$ cells. 100 μ l of the monoclonal phage antibody of the present invention was concentrated 10 times by PEG, followed by dilution at 1:2. The dilution was

mixed and stirred with the cells. The mixture was reacted in ice for 1 hour, followed by centrifugation at 1300 rpm at 4°C for 3 minutes to remove a supernatant. The precipitate was washed three times with 200 μl of a 2% PBF solution. 100 μl of anti-g8p antibody (Abcam) diluted at 1:200 in a 2% PBF solution was mixed and stirred with the resulting solution, followed by reaction in ice for 30 minutes. The reactant was centrifuged at 1300 rpm at 4°C for 3 minutes for removal of a supernatant, followed by washing three times with 200 μl of a 2% PBF solution. 100 μl of FITC-linked anti-mouse IgG diluted at 1:1000 in a 2% PBF solution was mixed with each specimen, followed by reaction in ice for 30 minutes. After a washing was additionally performed, 500 μl of a 2% PBF solution was added into it. The mixture was transferred to a tube for FACS (Falcon) and vortexed, followed by analysis of stained cells by flow cytometer (Beckman Coulter). In each experiment, monoclonal phage antibodies were treated with a specimen under the same conditions and used as an internal control group. WINMDI2.9 software (<http://facs.scripps.edu/software.html>, The Scripps Research Institute) was used to analyze the data.

As a result, as shown in FIG. 9, it was confirmed that C2 phage antibodies of the present invention specifically bound to ENO1 in various cancer cells like commercially available antibodies.

<6-3> Analysis of whole IgG conversion

To covert monoclonal phage antibodies against ENO1 into whole IgG vectors in phages, 1 μ l of monoclonal DNA, 10 pmole/ μ l of each of heavy chain forward primer and reverse primer in Table 8, 5 μ l of 10 $\times$ buffer, 1 μ l of 10 mM dNTP mix, 0.5 μ l of pfu DNA polymerase (Solgent, 2.5 U/ μ l), and distilled water were mixed to perform a colony PCR (iCycler iQ, BIO-RAD). In addition, light chain forward and reverse primers in Table 9 were used to perform a colony PCR by the same manner.

10

【Table 9】

Clone Name	Heavy Chain				Light Chain			
	Forward primer (Sfi I)		Reverse primer (Nhe I)		Forward primer (Sfi I)		Reverse primer (Bgl II)	
NO1-C2	NATVH 3-2: SEQ ID No. 55	TTGGTGGCC ACAGCGGCC GATGTCCAC TCGCAGGTG CAGCTGGTG GAGTC	NATJH-ALL: SEQ ID No. 57	GAG GAG GCT AGC TGA GGA GAC GGT GA	NATVL9: SEQ ID No. 58	TTGGTGGCCA CAGCGGCCGA TGTCCACTCG AATTTTATGC TGACTCAGCC	NATJL 1-R: SEQ ID No. 60	GAGGAGA GATCTTA GGACGGT GACCTTG GTCCC
	NATVH 9-1: SEQ ID No. 56	TTGGTGGCC ACAGCGGCC GATGTCCAC TCGCAGCTG GTACAGTCT GGAGCA			NATVL7: SEQ ID No. 59	TTGGTGGCCA CAGCGGCCGA TGTCCACTCG TCCTATGAGC TGACTCAGC	NATJL 2-R: SEQ ID No. 61	GAGGAGA GATCTTA GGACGGT CAGCTTG GTCCC

After a heavy chain gene obtained through PCR was purified with DNA-gel extraction kit (Qiagen), 1 μ l of pNATAB H vector (FIG. 11a) (10 ng), 15 μ l of heavy chain (100-200

ng), 2 μ l of 10 $\times$ buffer, 1 μ l of ligase (1 U/ μ l), and distilled water were mixed with the gene and left still at room temperature for 1-2 hours for linkage to the vector. The vector was left still in ice for 30 minutes along with a cell
5 for transformation (XL1-blue), followed by heat shock at 42 $^{\circ}$ C for 90 sec for transfection. It was again left still in ice for 5 minutes and 1 ml of LB medium was injected, followed by incubation at 37 $^{\circ}$ C for 1 hour. The mixture was smeared in LB Amp solid medium, followed by incubation at 37 $^{\circ}$ C for 16 hours.
10 Single colony was inoculated into 5 ml of LB Amp liquid medium, followed by incubation at 37 $^{\circ}$ C for 16 hours. A DNA-prep kit (Nuclogen) was used for the medium to extract a DNA.

In addition, pNATAB L vector (FIG. 11b) was used by the same manner to extract a DNA of the light chain.

15 Sequencing of the obtained DNA was performed by using a CMV-proF primer (SEQ ID No. 62: AAA TGG GCG GTA GGC GTG) (Solgent).

As a result, it was confirmed that the sequences of heavy and light chains of the 2 clone phages against ENO1 converted
20 into whole IgG were identical to those of the phage antibodies.

<6-4> Verification of whole IgG

40 μ g of PEI (Cat# 23966, Polysciences, Inc) and 10 μ g of
25 each antibody heavy chain DNA and light chain DNA in the whole

form were added into 293E cells (Invitrogen) for co-transfection to obtain a supernatant, which was identified by Western blot. Normal human IgG (Jackson Lab) was used as a control group.

5 As a result, as shown in FIG. 10, it was confirmed that ENO1-C2 and ENO1-B8 were successfully converted into whole IgG form compared to a control group.

10 Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

【CLAIMS】**【Claim 1】**

AN ENO1-specific human antibody comprising a heavy chain comprising a heavy chain variable region (V_H) comprising a heavy chain complementarity determining region (HCDR) 1 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 7 to 12, HCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 13 to 18, and HCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 19 to 24, or a fragment thereof; and

a light chain comprising a light chain variable region (V_L) comprising a light chain complementarity determining region (LCDR) 1 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 31 to 36, LCDR 2 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 37 to 42, and LCDR 3 having an amino acid sequence selected from the group consisting of SEQ ID Nos. 43 to 48, or a fragment thereof.

【Claim 2】

The human antibody as set forth in claim 1, wherein the heavy chain variable region has an amino acid sequence selected from the group consisting of SEQ ID Nos. 25 to 30.

【Claim 3】

The human antibody as set forth in claim 1, wherein the light chain variable region has an amino acid sequence selected from the group consisting of SEQ ID Nos. 49 to 54.

5

【Claim 4】

A polynucleotide encoding a heavy chain of the human antibody of claim 1 or an immunologically active fragment thereof.

10

【Claim 5】

A polynucleotide encoding a light chain of the human antibody of claim 1 or an immunologically active fragment thereof.

15

【Claim 6】

An expression vector comprising the polynucleotide of claim 4.

20 **【Claim 7】**

An expression vector comprising the polynucleotide of claim 5.

【Claim 8】

25 A transformant prepared by introducing the expression vector of claim 6 into a host cell.

【Claim 9】

A transformant prepared by introducing the expression vector of claim 7 into a host cell.

5

【Claim 10】

A transformant prepared by introducing the expression vectors of claims 6 and 7 simultaneously into a host cell.

10 **【Claim 11】**

A method for preparing an ENO1-specific human antibody, the method comprising:

- 1) incubating the transformant of claim 10; and
 - 2) purifying the human antibody of claim 1 from the
- 15 medium.

【Claim 12】

A composition comprising the human antibody of claim 1.

20 **【Claim 13】**

A pharmaceutical composition comprising the human antibody of claim 1.

【Claim 14】

25 The pharmaceutical composition as set forth in claim 13,

wherein the pharmaceutical composition is used to prevent or treat an ENO1-overexpressed cancer.

【Claim 15】

5 The pharmaceutical composition as set forth in claim 14, wherein the ENO1-overexpressed cancer is selected from the group consisting of lung cancer, colorectal cancer, breast cancer, ovarian cancer, renal cell cancer, and prostate cancer.

10

【Claim 16】

The pharmaceutical composition as set forth in claim 13, wherein the pharmaceutical composition is administered in combination with chemotherapy.

15

【Claim 17】

A method for treating an ENO1-overexpressed cancer, the method comprising administering a pharmaceutically effective amount of the human antibody of claim 1 to a subject with the cancer.

20

【Claim 18】

The method as set forth in claim 17, wherein the ENO1-overexpressed cancer is selected from the group consisting of lung cancer, colorectal cancer, breast cancer, ovarian cancer, renal cell cancer, and prostate cancer.

25

【Claim 19】

A composition comprising the human antibody of claim 1, a light or heavy chain of the human antibody or an immunologically active fragment thereof, and a radioactive isotope.

【Claim 20】

The composition as set forth in claim 19, wherein the composition is used for radioimmuno treatment or detection of an ENO1-overexpressed cancer.

【Claim 21】

The composition as set forth in claim 20, wherein the ENO1-overexpressed cancer is selected from the group consisting of lung cancer, colorectal cancer, breast cancer, ovarian cancer, renal cell cancer, and prostate cancer.

【Claim 22】

The composition as set forth in claim 19, wherein the radioactive isotope is selected from the group consisting of ^3H , ^{11}C , ^{14}C , ^{18}F , ^{64}Cu , ^{76}Br , ^{86}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , ^{177}Lu , and a mixture or combination thereof.

【Claim 23】

The composition as set forth in any one of claims 19 to 22, wherein the radioactive isotope is bound to a human antibody or included in a carrier to which the human antibody
5 is bound.

【Claim 24】

An immunodetection method for detecting an *ex vivo* ENO1-overexpressed cancer, the method comprising contacting the
10 composition of claim 19 with a cancer cell.

【Claim 25】

A method for imaging an *in vivo* ENO1-overexpressed cancer, the method comprising:

- 15 1) administering a diagnostically effective amount of the composition of claim 19 to a subject; and
 2) obtaining a detection image for the subject.

【Claim 26】

20 The method as set forth in claim 25, wherein the detection image is obtained by near-infrared light imaging, PET, MRI, or ultrasonic imaging.

【Claim 27】

A method for treating an *in vivo* ENO1-overexpressed cancer, the method comprising:

- 1) intravenously administering a composition for
5 detection of claim 19 to a subject;
- 2) detecting the composition of Step 1) to identify tumor cells; and
- 3) eliminating the tumor cells identified in Step 2) by surgical operation.

10

【Claim 28】

A method for prognostic evaluation of a cancer patient, the method comprising:

- 1) intravenously administering a composition of claim 19
15 to a patient whose tumor has been eliminated;
- 2) detecting the composition of Step 1) to identify tumor cells; and
- 3) judging that all tumor cells have been eliminated when tumor cells are not detected in step 2).

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Figures
Fig. 1

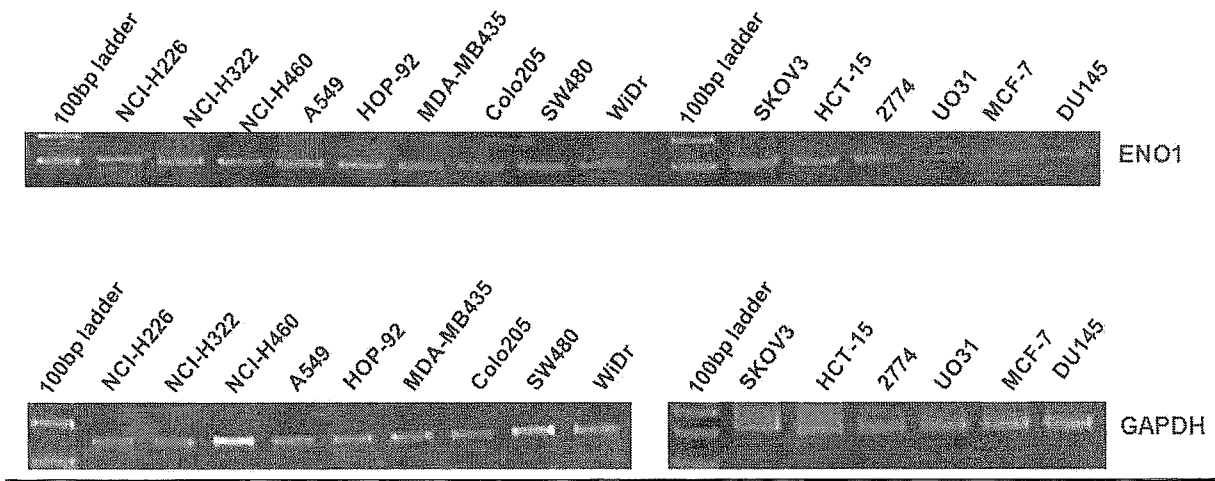
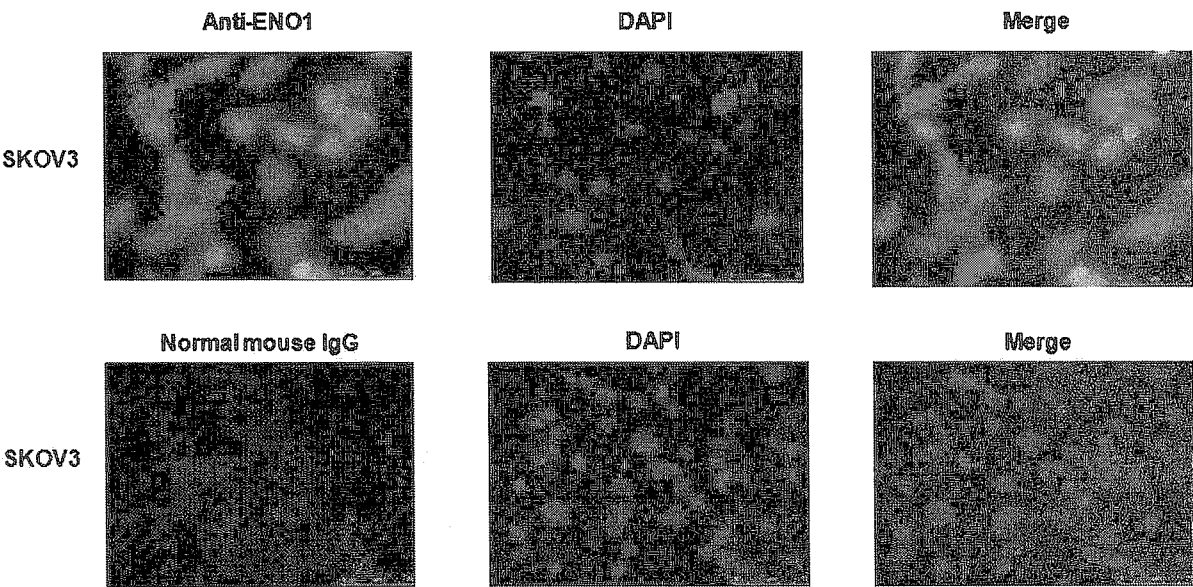
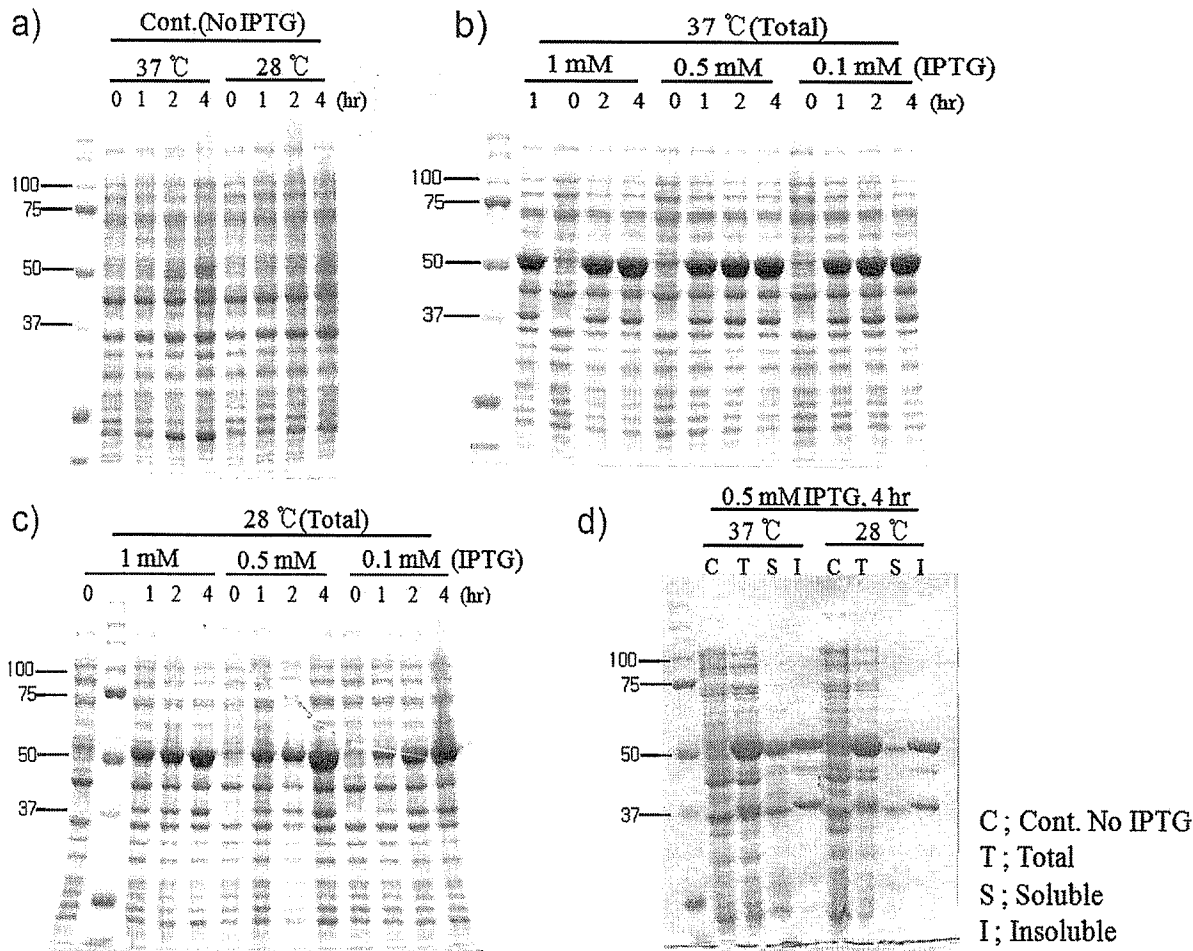


Fig. 2



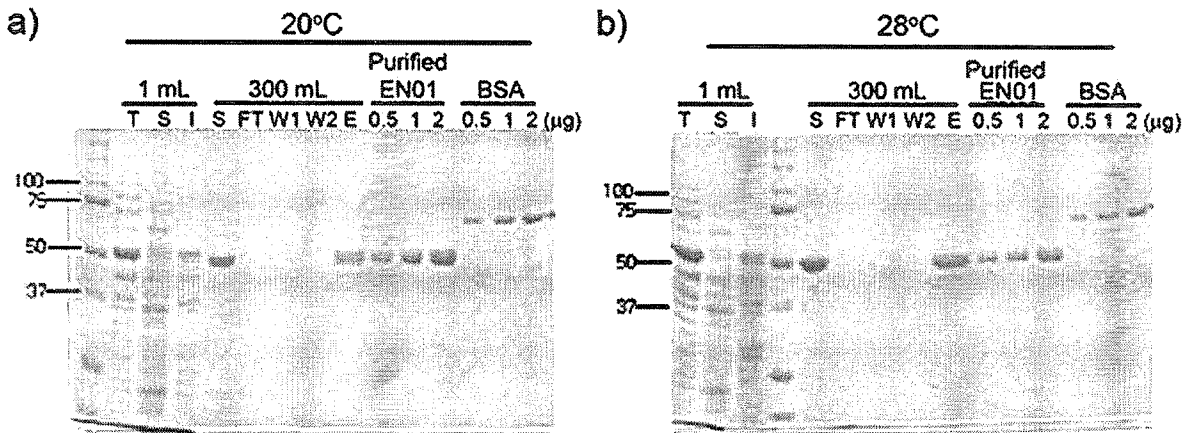
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Fig. 3



EN01 induction test in BL21(DE3)

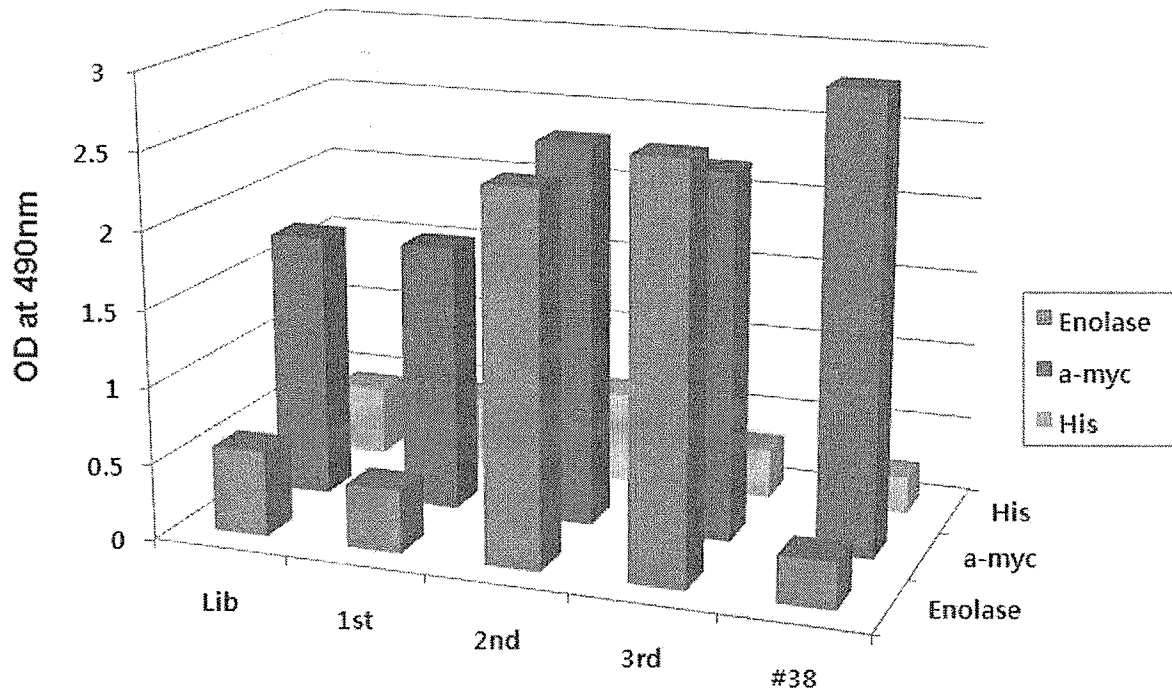
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Fig. 4



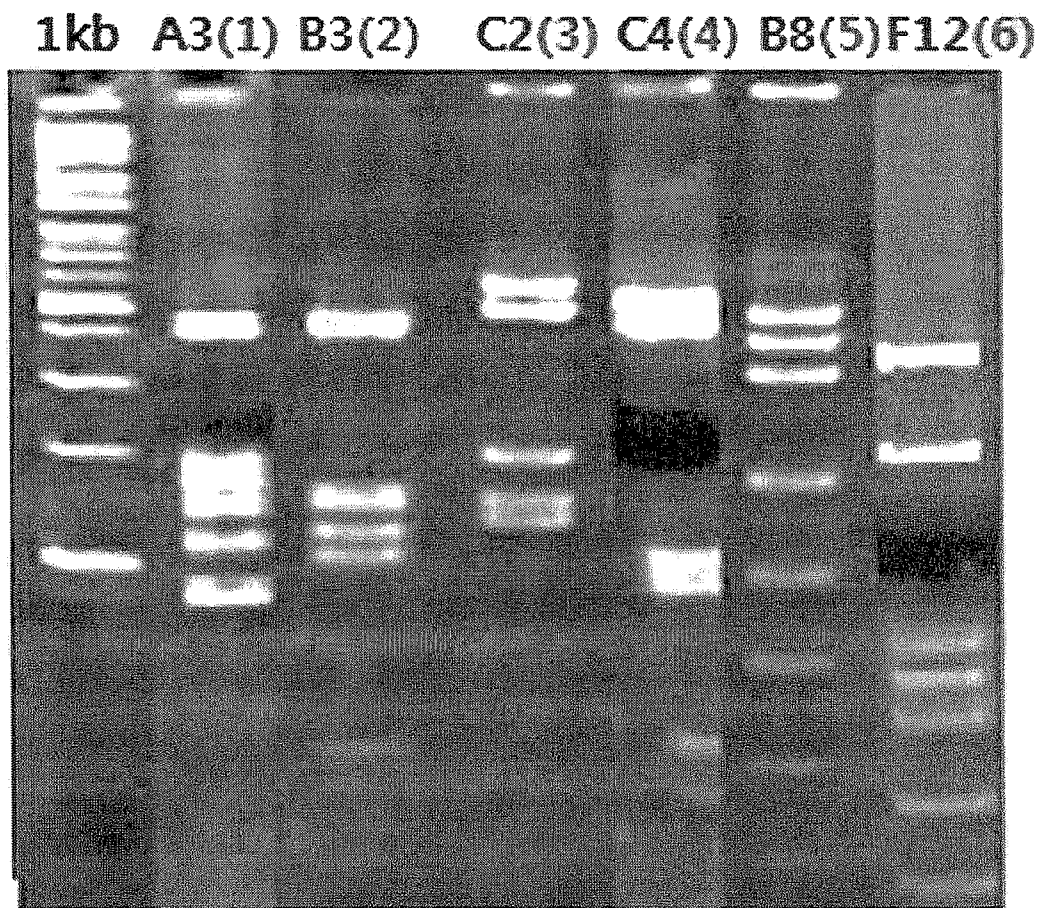
T : Total lysate
S : Soluble form
I : Insoluble form
FT: Flow-through
W1: Wash 1
W2: Wash 2
E: Elute

Fig. 5



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Fig. 6



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Fig. 7a

Heavy Chain

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----- FR 1 ----- CDR1 ----- FR 2 -----
1
ENO1-A3 (1) MA QVQLVQSGAEVKKPGESLKISCKGSGYNFN NHWIG WVRQMPGKGLEWMG
ENO1-B3 (1) MA QVQLVESGAEVKKPGESLRISCKASGYRFS SYWLT WVRQIPGEGGLEWMG
ENO1-C2 (1) MA QVQLVESGAEVKKPGQSLTISCKGSGYIFT SNWIS WVRQMPGKGLEWMG
ENO1-C4 (1) MA QVQLVESGGGLVQPGMSLRSLSCAASGFTFD EYAMH WVRQAPGKGLEWVS
ENO1-B8 (1) MA QMQLVQSGAEVKKPGESLKISCEASGYNFN THWIG WVRQMPGKGLEWMG
ENO1-F12 (1) MA QVQLVESGGGVAQPGRLRLSCGASQFVFS NYDMH WVRQAPGKGLEWLA

----- CDR 2 ----- FR 3 -----
51
ENO1-A3 (51) IIHPRDS DTRYSPAFQG QVTMSADKSISTAYLQWSSLKASDTAMYYCAR
ENO1-B3 (51) RIDPNDSFSLYNPAFQG RVTMSVDKSTSTAYLQWTSCLKASDTAMYYCAR
ENO1-C2 (51) AIDPLDSYTNYSPSFQG HVTLTVDKSVSTAYLQWDSLKASDTAMYYCAR
ENO1-C4 (51) HISWNSGSIAYGDSVKG RFTISRDNAKNSLYLQMNLSRAEDTAVYYCAR
ENO1-B8 (51) IIHPGNSDIRYSPSFQG QVTFSADKSIGTAYLQWNSCLKASDTAMYYCAR
ENO1-F12 (51) LIAYDGSYQHHADSVQA RFTISRDNRSNTLFLQMNLSRSED TAVYYCAN

----- CDR 3 ----- FR 4----- Linker -----
101
ENO1-A3 (101) HKVSGNSWYSGSLAY WQGGTLVTVSS GLGGLGGGGSGGGSGGSSGVGS S (SEQ ID No. 25)
ENO1-B3 (101) QHN-GNGDLLNALDI WQGGTAVTVSS GLGGLGGGGSGGGSGGSSGVGS S (SEQ ID No. 26)
ENO1-C2 (101) SNQ-----ATFDV WQGGTMITVSS GLGGLGGGGSGGGSGGSSGVGS N (SEQ ID No. 27)
ENO1-C4 (101) GS GSGIYYKNYGMDV WQGGTTVTVSS GLGGLGGGGSGGGSGGSSGVGS S (SEQ ID No. 28)
ENO1-B8 (101) NSDIGTNRRMG-FDI WQGGTMITVSS GLGGLGGGGSGGGSGGSSGVGS S (SEQ ID No. 29)
ENO1-F12 (101) WNWG-----LGFDF WGRGTLVTVSS GLGGLGGGGSGGGSGGSSGVGS D (SEQ ID No. 30)

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Fig. 7b

Light Chain

	----- FR 1 -----	--- CDR 1 ---	-----FR2-----
	151		200
ENO1-A3 (151)	YELTQ-PLSVSMSPGQTARITC	SG-DALSKQYAS	WYQLKPGQAPVVVMY
ENO1-B3 (150)	YELTQ-PPSVSVSPGQTATITC	SG-DVLSKQYAY	WYQQKPGQAPVLVIY
ENO1-C2 (144)	FMLTQ-PPSASGTPGQRITISC	SGTSSNIGNNFVY	WYQQVPGTAPKLLIY
ENO1-C4 (151)	YELTQ-APSLVSPGQTANIIC	SG-DNLRTKYVS	WYQQKPGQSPLLVIY
ENO1-B8 (150)	YELTQ-PLSVSLSPGQTARITC	SG-DALSKEYSY	WYQQKPGQAPVLVMY
ENO1-F12 (145)	IVMTQTPDFQAVTPNEKVTITC	RA-SQNIANS�H	WYQQKPDQSPVLLIK

	-CDR 2-	FR 3	--- CDR3---
	201		250
ENO1-A3 (198)	KDTERPL	GIPDRFSGSSSGTTVTLTISGVQAEDEADYYC	QSITDKSGTDV
ENO1-B3 (197)	KDNVRPS	GIPERFSGSSSGTTVTLTISGVQAEDEGDYFC	QSGDRSGSYRV
ENO1-C2 (193)	RNDQRPS	GVPDRFSGSTSGTSASLAISGLRSEDEADYYC	ATW-DDSLSGY
ENO1-C4 (198)	QDTRRPS	GIPARFSGSMSGNTATLTISGTQTRDESTYYC	MTW-DVDTTSM
ENO1-B8 (197)	KDSERPS	GIPERFSGSSSGTTVTLTISGVQAEDEADYYC	QSV-DSSDTSV
ENO1-F12 (193)	YASQSI	GVPSRFSGSGSGTEFTLTIIYSLEDEDVATYY	MQG-SSRPY

	--- FR 4 ---	----- Myc tag -----
	251	
ENO-A3 (248)	I	FGGGTNLTVL GGGASLVEFEQKLISEEDL- (SEQ ID No. 49)
ENO-B3 (247)	L	FGGGTKLTVL GGGASLVEFEQKLISEEDL- (SEQ ID No. 50)
ENO-C2 (242)	V	FATGTKVTVL GGGASLVEFEQKLISEEDL- (SEQ ID No. 51)
ENO-C4 (247)	I	FGGGTKLTVL GGGASLVEFEQKLISEEDL- (SEQ ID No. 52)
ENO-B8 (246)	V	FGGGTKLTVL GGGASLVEFEQKLISEEDL- (SEQ ID No. 53)
ENO-F12 (241)	I	FGQGTKLEIK RGGASLVEFEQKLISEEDL- (SEQ ID No. 54)

Fig. 8

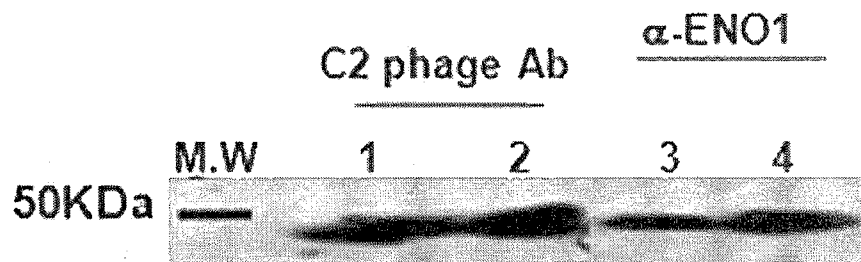
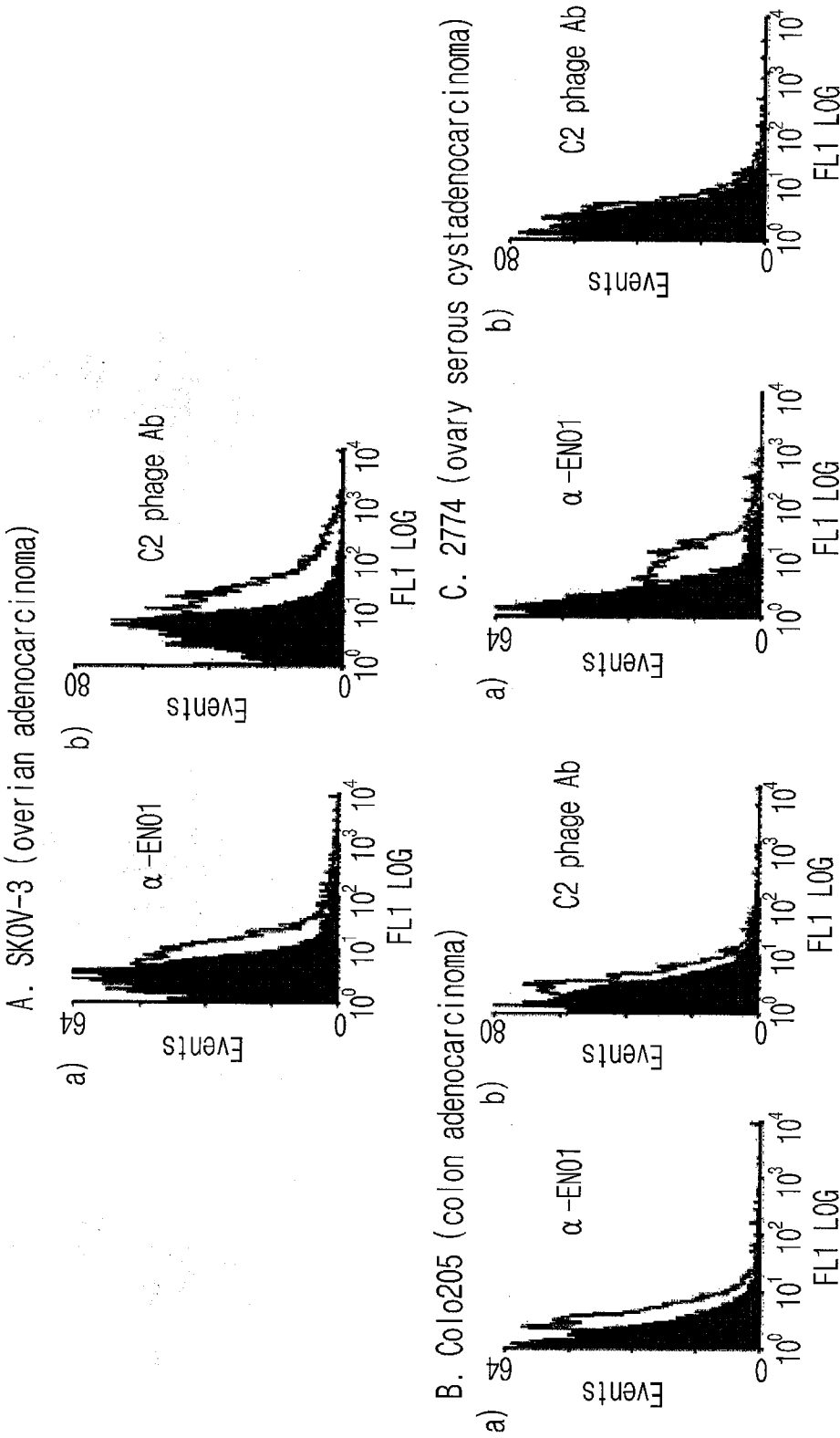
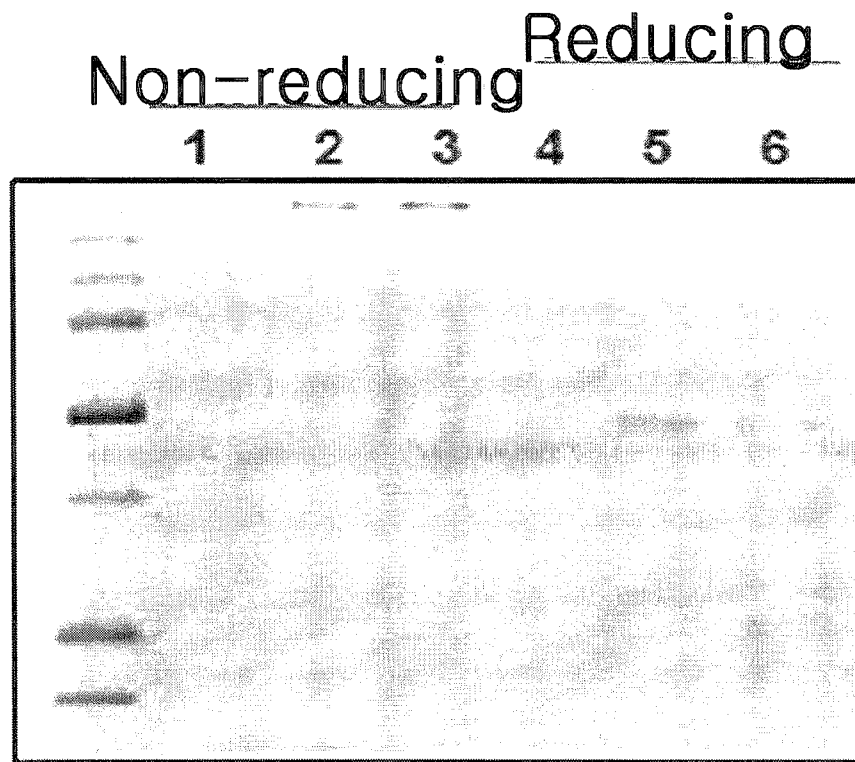


Fig. 9



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Fig. 10



Lane1 . human IgG 1ug

Lane 2. purified EN B8 1ug

Lane3 . purified EN C2 1ug

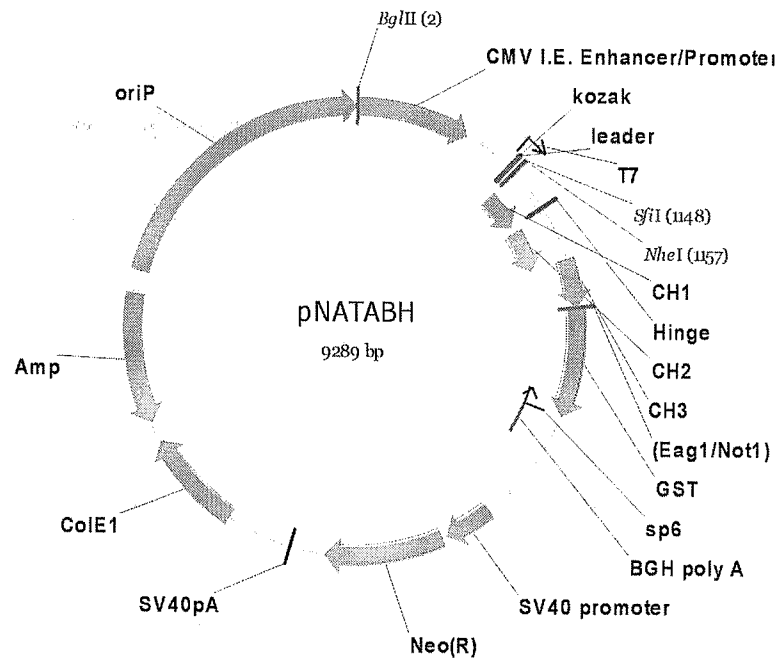
Lane4. human IgG 1ug

Lane5. purified EN B8 1ug

Lane6. purified EN C2 1ug

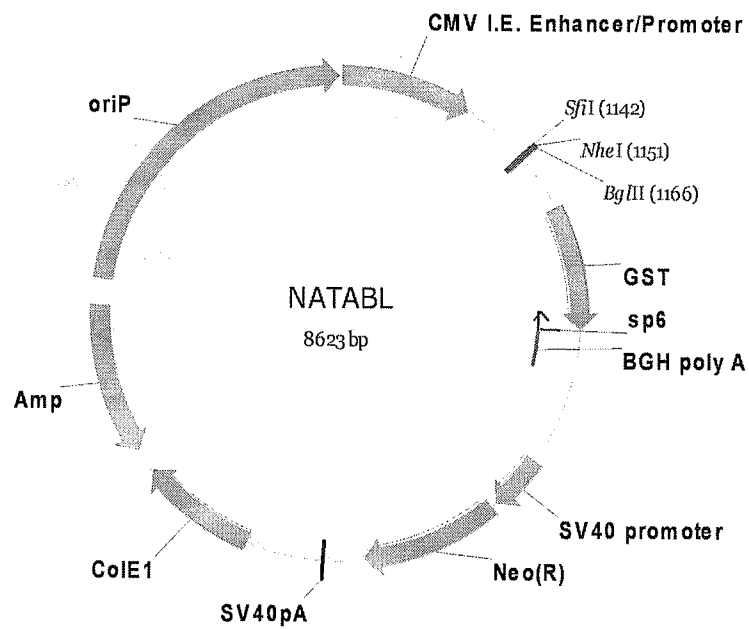
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Fig. 11a



H chain vector

Fig. 11b



L chain vector