

DESCRIPTION

PHARMACEUTICAL COMPOSITION CONTAINING TAGGED SITE-ANTIHUMAN ANTIBODY FAB FRAGMENT COMPLEX

TECHNICAL FIELD

[0001] The present invention relates to a stable pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate. Particularly, the present invention relates to a stable pharmaceutical composition comprising a labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate or a labeling moiety-anti-human MUC1 antibody Fab fragment conjugate. The present invention also relates to a method for producing a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, and a method for stably preserving a labeling moiety-anti-human antibody Fab fragment conjugate.

BACKGROUND ART

[0002] Development of genetic modification technology has enabled use of antibodies such as immunoglobulin, monoclonal antibodies and humanized antibodies as medicinal products. For example, for diagnosing and treating cancers, antibodies bound to anticancer agents, metal radioisotopes, fluorescent dyes and the like are used. Targeting using an antibody is known to have high specificity to tumor cells and cause little side-effects. Under such circumstances, monoclonal antibodies labeled with metal radioisotopes, and the like have been heretofore developed (Patent literature 1).

[0003] Meanwhile, antibodies generally have a long half-life in blood and require a period as long as 4 days to 5 days for reaching a tumor-to-blood ratio that confers a signal-to-background ratio sufficient for visualizing a cancer, after administration into the body (Clin. Pharmacol. Ther.; 2010; 87: 586-592). Also, the Fc regions of antibodies cause a pharmacological effect of antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) (Glycoconj. J.; 2013; 30: 227-236; and Curr. Opin.

Biotechnol.; 2002; 13: 609-614). Furthermore, antibodies are metabolized in the liver and therefor highly accumulate in the liver, regardless of a target. However, it is difficult to detect lesions of hepatic metastasis of colorectal cancer using antibodies because the early metastasis of colorectal cancer is localized to the liver (Clin. Pharmacol. Ther.; 2010; 87: 586-592).

[0004] On the other hand, low-molecular recombinant antibody fragments such as Fab easily arrive at lesions because of their high tissue penetration, and production at low cost using an expression system in *E. coli* or yeasts can be expected. Utilization of the low-molecular recombinant antibody fragments as a diagnostic drug is expected because of their short half-lives in blood and the feature of renal excretion (Nat. Biotechnol.; 2005; 23: 1126-1136).

[0005] Under such circumstances, studies have been made on utilization of Fab fragment conjugates in which a metal radioisotope is coordinated and Fab fragments bound to a fluorescent dye for the purpose of diagnosing a cancer.

[0006] Many studies have been heretofore made on methods for stably preserving antibodies. For example, Patent Literature 2 discloses a method in which a nonionic surfactant and a saccharide are added to a formulation containing a humanized C4C1 Fab fragment, and the pH is adjusted to a specific range to achieve stabilization. Further, Patent Literature 3 discloses a method in which a buffering agent is added to a formulation comprising a human antibody to IL-1 β , and the pH is adjusted to a specific range to achieve stabilization.

CITATION LIST

PATENT LITERATURE

[0007]

PTL 1: Japanese Translation of PCT International Application Publication No. 2013-510093

PTL 2: International Publication No. WO 00/66160

PTL 3: Japanese Translation of PCT International Application Publication No. 2012-511540

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0008] Monovalent Fab fragments have a molecular weight of approximately 50 kDa, which is smaller than that (approximately 150 kDa) of antibodies, undergo renal excretion, and also have a short half-life in blood. They lack a Fc region and therefore cause neither ADCC nor CDC. From these features, Fab fragments in which a metal radioisotope is coordinated or Fab fragments bound to a fluorescent dye are expected to be more effective as diagnostic drugs as compared with antibodies.

[0009] However, Fab fragments bound to a ligand or a fluorescent dye have the problem that multimers or insoluble subvisible particles are easily generated due to heat stress or light stress during preservation and the problem that insoluble subvisible particles are generated in the process of thawing and stirring before use. Addition of various compounds into a preservation solution for stabilization may cause problems of coordination efficiency and fluorescence color degradation.

[0010] An object of the present invention is to provide a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, which enables suppression of generation of multimers or insoluble subvisible particles during preservation, and the like. Another object of the present invention is to provide a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, which enables suppression of a decrease in coordination efficiency of a metal radioisotope to a ligand when the ligand is used as a labeling moiety. Still another object of the present invention is to provide a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, which enables suppression of fluorescent dye color degradation when a fluorescent dye is used as a labeling moiety.

SOLUTION TO PROBLEM

[0011] The present inventors have conducted considerable diligent studies on the

formulation of a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, and consequently found that by adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent, adding sucrose or glycerin as a stabilizer, and further adding a nonionic surfactant to prepare a pharmaceutical composition having a pH of 6.5 to 7.5, generation of multimers or insoluble subvisible particles during preservation of the labeling moiety-anti-human antibody Fab fragment conjugate can be suppressed, and a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation can be suppressed. In this way, the present invention has been achieved.

[0012] Specifically, the present invention provides a labeling moiety-anti-human antibody Fab fragment conjugate, which is excellent in preservation stability of the labeling moiety-anti-human antibody Fab fragment conjugate and enables suppression of a decrease in coordination efficiency of metal to the labeling moiety and labeling fluorescent dye color degradation. In one embodiment, the present invention may be as follows.

[0013] [1] A pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, a buffering agent, a stabilizer and a nonionic surfactant and having a pH of 6.5 to 7.5,

the buffering agent comprising citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane,

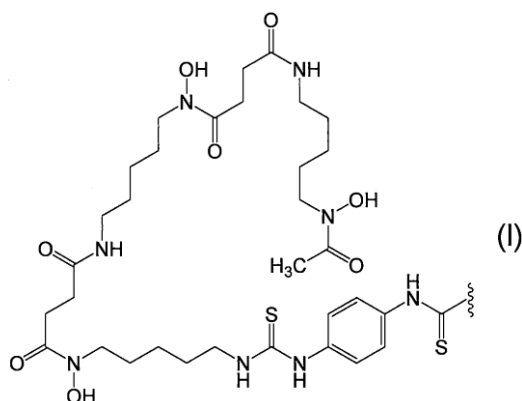
the stabilizer comprising sucrose or glycerin.

[2] The pharmaceutical composition according to [1], wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4, and the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 1]



wherein the wavy line represents binding to the anti-human CEACAM5 antibody Fab fragment, where the anti-human CEACAM5 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human CEACAM5 antibody Fab fragment.

[3] The pharmaceutical composition according to [2], wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO:

2 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

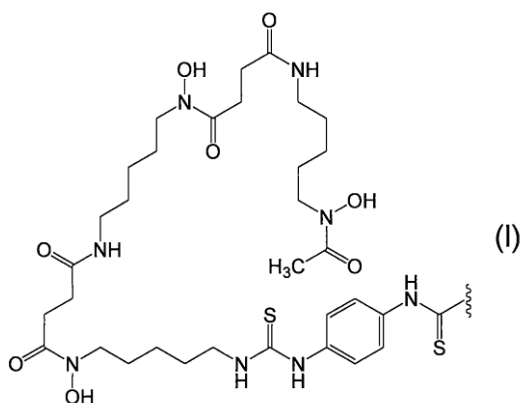
[4] The pharmaceutical composition according to [1], wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and

(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and

the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 2]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human MUC1 antibody Fab fragment.

[5] The pharmaceutical composition according to [4], wherein the anti-human antibody Fab

fragment is one or more selected from the group consisting of the following (a) and (b):

- (a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and
- (b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[6] The pharmaceutical composition according to any one of [1] to [5], wherein the nonionic surfactant comprises Polysorbate 80.

[7] The pharmaceutical composition according to any one of [2] to [6], wherein the labeling moiety-anti-human antibody Fab fragment conjugate further comprises ⁸⁹Zr.

[8] The pharmaceutical composition according to [7] for use in the diagnosis of colorectal cancer or a cancer resulting from the metastasis of colorectal cancer.

[9] The pharmaceutical composition according to [7] for use in the diagnosis of breast cancer or a cancer resulting from the metastasis of breast cancer.

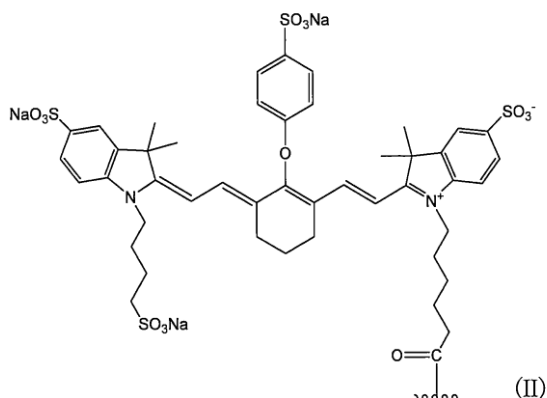
[10] The pharmaceutical composition according to [1], wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

- (a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and
- (b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region

consisting of the amino acid sequence represented by SEQ ID NO: 16, and

the labeling moiety is a group represented by the following formula (II):

[Chemical Formula 3]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=O) group via an amino group in the anti-human MUC1 antibody Fab fragment.

[11] The pharmaceutical composition according to [10], wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and
(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[12] The pharmaceutical composition according to [10] or [11], wherein the nonionic surfactant comprises Polysorbate 80.

[13] The pharmaceutical composition according to any one of [10] to [12] for use in the diagnosis of breast cancer or a cancer resulting from the metastasis of breast cancer.

[14] The pharmaceutical composition according to [13], which is an intraoperative diagnostic drug.

[15] The pharmaceutical composition according to any one of [1] to [14], wherein the concentration of the buffering agent is 10 to 30 mmol/L.

[16] The pharmaceutical composition according to any one of [1] to [15], wherein the concentration of the stabilizer is 5 to 30 w/v%.

[17] The pharmaceutical composition according to any one of [1] to [16], wherein the concentration of the nonionic surfactant is 0.02 to 0.2 w/v%.

[18] The pharmaceutical composition according to any one of [1] to [17], which is a liquid formulation, a frozen formulation or a lyophilized formulation.

[19] A method for producing a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of:

- (a) producing and adding the labeling moiety-anti-human antibody Fab fragment conjugate;
- (b) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent;
- (c) adding sucrose or glycerin as a stabilizer;
- (d) adding a nonionic surfactant; and
- (e) adjusting the pH to 6.5 to 7.5.

[20] A method for stably preserving a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of:

- (a) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent to a solution containing the labeling moiety-anti-human antibody Fab fragment conjugate;
- (b) adding sucrose or glycerin as a stabilizer to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate;
- (c) adding a nonionic surfactant to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate; and
- (d) adjusting the pH of the solution containing the labeling moiety-anti-human antibody Fab

fragment conjugate to 6.5 to 7.5.

ADVANTAGEOUS EFFECTS OF DRAWINGS

[0014] The pharmaceutical composition of the present invention is useful in terms of stability during storage, transportation and use because it enables suppression of generation of multimers or insoluble subvisible particles during preservation of the labeling moiety-anti-human antibody Fab fragment conjugate, and enables suppression of a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation. The pharmaceutical composition of the present invention is also useful in terms of safety in administration to a human because pharmaceutically acceptable buffering agents and medicinal additives are used in consideration of safety.

DESCRIPTION OF EMBODIMENTS

[0015] Hereinafter, the present invention will be described in detail. However, the present invention is not limited thereby. Scientific terms and technical terms used in relation to the present invention have meanings generally understood by those skilled in the art, unless otherwise specified herein.

[0016] 1. Pharmaceutical composition

In a certain embodiment, the present invention relates to a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, a buffering agent, a stabilizer and a nonionic surfactant and having a pH of 6.5 to 7.5, the buffering agent comprising citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane, the stabilizer comprising sucrose or glycerin. The pharmaceutical composition enables suppression of generation of multimers or insoluble subvisible particles during preservation of the labeling moiety-anti-human antibody Fab fragment conjugate, and enables suppression of a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation. Thus, for the pharmaceutical composition of the present invention,

aggregation etc. during storage, transportation and use of the labeling moiety-anti-human antibody Fab fragment conjugate can be suppressed.

[0017] 1-1. Anti-human antibody Fab fragment

The basic structure of an antibody molecule is common among classes and is constituted by heavy chains having a molecular weight of 50000 to 70000 and light chains having a molecular weight of 20000 to 30000. The heavy chain usually consists of a polypeptide chain comprising approximately 440 amino acids, has a structure characteristic of each class, and is called γ , μ , α , δ , and ϵ chains corresponding to IgG, IgM, IgA, IgD, and IgE. IgG further has IgG1, IgG2, IgG3, and IgG4 which are called γ_1 , γ_2 , γ_3 , and γ_4 , respectively. The light chain usually consists of a polypeptide chain comprising approximately 220 amino acids and is known as two types, L and K types, which are called λ and κ chains, respectively. As for the peptide configuration of the basic structure of the antibody molecule, two homologous heavy chains and two homologous light chains are linked through disulfide bonds (S-S bonds) and non-covalent bonds to form a molecular weight of 150000 to 190000. The two light chains can pair with any of the heavy chains. An individual antibody molecule is constantly made up of two identical light chains and two identical heavy chains.

[0018] Four (or five for μ and ϵ chains) and two intrachain S-S bonds are present in the heavy chain and the light chain, respectively, and each constitute one loop per 100 to 110 amino acid residues. This conformation is similar among the loops and is called structural unit or domain. For both the heavy chain and the light chain, a domain positioned on the N-terminal side does not have a constant amino acid sequence even among preparations from the same classes (subclasses) of animals of the same species, and is thus called variable region. The respective domains are called heavy chain variable region (V_H domain) and light chain variable region (V_L domain). An amino acid sequence on the C-terminal side therefrom is almost constant on a class or subclass basis and called constant region. The respective domains are represented by C_{H1} , C_{H2} , C_{H3} and C_L .

[0019] The binding specificity of the antibody for an antigen depends on the amino acid

sequence of a moiety constituted by the heavy chain variable region and the light chain variable region. On the other hand, biological activity such as binding to complements or various cells reflects the difference in structure among the constant regions of Igs of respective classes. It is known that the variability of the heavy chain and light chain variable regions is limited substantially by three small hypervariable regions present in both the chains. These regions are called complementarity determining regions (CDRs; CDR1, CDR2, and CDR3 in order from the N-terminal side). The remaining moieties of the variable region are called framework regions (FRs) and are relatively constant.

[0020] A region between the C_{H1} domain and the C_{H2} domain of the heavy chain constant region of an antibody is called hinge region. This region is rich in proline residues and contains a plurality of interchain S-S bonds that connect two heavy chains. For example, the hinge regions of human IgG1, IgG2, IgG3, and IgG4 contain 2, 4, 11, and 2 cysteine residues, respectively, which constitute S-S bonds between the heavy chains. The hinge region is a region highly sensitive to a proteolytic enzyme such as papain or pepsin. In the case of digesting an antibody with papain, the heavy chains are cleaved at a position on the N-terminal side from the inter-heavy chain S-S bonds of the hinge region and thus decomposed into two Fab fragments and one Fc fragment. The Fab fragment is constituted by a light chain and a heavy chain fragment comprising a heavy chain variable region, a C_{H1} domain and a portion of the hinge region. The Fab fragment comprises variable regions and has antigen binding activity.

[0021] A certain embodiment of the anti-human antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human CEACAM5 antibody Fab fragment. A certain embodiment of the anti-human antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment.

[0022] 1-1-1. Anti-human CEACAM5 antibody Fab fragment

CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule 5) is one of tumor markers, and is rarely expressed in normal tissues, but is expressed in the fetal

gastrointestinal tract or colorectal cancer (BBA; 1990; 1032: 177-189; and Mol. Pathol.; 1999; 52: 174-178). CEACAM5 is known to be also expressed in breast cancer and the like (Diagn. Cytopathol.; 1993; 9: 377-382; Cancer Res.; 1990; 50: 6987-6994; Histopathology; 2000; 37: 530-535). The concentration of CEACAM5 in blood is higher in colorectal cancer patients than in healthy persons (J. Exp. Med.; 1965; 121: 439-462), and CEACAM5 is used as a tumor marker. According to the histological studies of colorectal cancer patients, CEACAM5 is highly expressed in 90% or more tissues (British J. Cancer; 2013; 108: 662-667).

[0023] The anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention includes a Fab fragment having the following feature: an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4.

[0024] Any constant region of Ig γ 1, Ig γ 2, Ig γ 3 or Ig γ 4, etc. can be selectable as the heavy chain constant region of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention. In one embodiment, the heavy chain constant region of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention is a human Ig γ 1 constant region.

[0025] Any constant region of Ig λ or Ig κ can be selectable as the light chain constant region of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention. In one embodiment, the light chain constant region of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention is a human Ig κ constant region.

[0026] In one embodiment, the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention is the following Fab fragment: an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment

consisting of the amino acid sequence represented by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0027] In the case of expressing an antibody comprising a Fab fragment in cells, the antibody is known to undergo a posttranslational modification. Examples of the posttranslational modification include the cleavage of heavy chain C-terminal lysine by carboxypeptidase, the modification of heavy chain and light chain N-terminal glutamine or glutamic acid into pyroglutamic acid by pyroglutamylation, glycosylation, oxidation, deamidation, and glycation. Such a posttranslational modification is known to occur in various antibodies (J. Pharm. Sci., 2008; 97: 2426-2447).

[0028] The anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention can also include a Fab fragment resulting from the posttranslational modification. Examples of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention that can result from the posttranslational modification include an anti-human CEACAM5 antibody Fab fragment having an N-terminally pyroglutamylated heavy chain. It is known in the art that such a posttranslational modification by N-terminal pyroglutamylation has no marked influence on the activity of the antibody (Anal. Biochem., 2006; 348: 24-39).

[0029] In one embodiment, the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human CEACAM5 antibody Fab fragment having the following feature:

an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4.

[0030] In another embodiment, the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human

CEACAM5 antibody Fab fragment having the following feature:

an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0031] The pharmaceutical composition of the present invention also includes an anti-human CEACAM5 antibody Fab fragment having the following feature:

an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region comprising CDR1 consisting of an amino acid sequence from amino acid positions 31 to 35 of SEQ ID NO: 2, CDR2 consisting of an amino acid sequence from amino acid positions 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of an amino acid sequence from amino acid positions 99 to 110 of SEQ ID NO: 2, and a light chain comprising a light chain variable region comprising CDR1 consisting of an amino acid sequence from amino acid positions 24 to 38 of SEQ ID NO: 4, CDR2 consisting of an amino acid sequence from amino acid positions 54 to 60 of SEQ ID NO: 4, and CDR3 consisting of an amino acid sequence from amino acid positions 93 to 101 of SEQ ID NO: 4.

[0032] The anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention binds to human CEACAM5. A method for measuring the binding activity of the obtained anti-human CEACAM5 antibody Fab fragment against human CEACAM5 includes methods such as analysis by surface plasmon resonance (SPR) and ELISA. In the case of using, for example, analysis by SPR, an association rate constant (k_a), a dissociation rate constant (k_d), and a dissociation constant (K_D) can be measured by using Biacore T200 (GE Healthcare Japan Corp.), immobilizing Biotin CAPture Kit (GE Healthcare Japan Corp.) and biotinylated human CEACAM5 onto a sensor chip, and adding a serially diluted Fab fragment thereto.

[0033] The anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention can be readily prepared by those skilled in the art using

a method known in the art on the basis of sequence information on the heavy chain fragment and the light chain of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention disclosed herein. The anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention can be produced according to, but not particularly limited to, a method described in, for example, <4-4. Method for producing anti-human antibody Fab fragment> mentioned later.

[0034] 1-1-2. Anti-human MUC1 antibody Fab fragment

Mucin 1 (MUC1) is a membrane-bound glycoprotein that is expressed on the lumen side of epithelial cells constituting the epithelial tissues of the mammary gland, the trachea and the gastrointestinal tract, etc. (Nat. Rev. Cancer, 2004 Jan; 4 (1): 45-60). MUC1 is overexpressed in cancer cells of breast cancer and colorectal cancer (Mod. Pathol.; 2005 Oct; 18 (10): 1295-1304, Int. J. Oncol.; 2000 Jan; 16 (1): 55-64). MUC1 is useful as a target molecule for detecting a cancer focus (Nat. Rev. Cancer; 2004 Jan; 4 (1): 45-60; and Pathol. Res. Pract.; 2010 Aug 15; 206 (8): 585-9).

[0035] The anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is a Fab fragment having the following feature:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0036] In one embodiment, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0037] Any constant region of Ig γ 1, Ig γ 2, Ig γ 3 or Ig γ 4, etc. can be selectable as the heavy

chain constant region of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention. In one embodiment, the heavy chain constant region of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is a human Ig γ 1 constant region.

[0038] Any constant region of Ig λ or Ig κ can be selectable as the light chain constant region of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention. In one embodiment, the light chain constant region of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is a human Ig κ constant region.

[0039] In one embodiment, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is the following Fab fragment:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0040] In one embodiment, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0041] In the case of expressing an antibody comprising a Fab fragment in cells, the antibody is known to undergo a posttranslational modification as described above. Thus, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention can also include a Fab fragment resulting from the posttranslational modification. Examples of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention resulting from the posttranslational modification include an anti-human MUC1 antibody Fab fragment having an N-terminally pyroglutamylated heavy chain. It is known in the art that such a posttranslational modification by N-terminal pyroglutamylation has no influence on the activity of the

antibody as described above.

[0042] In one embodiment, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment having the following feature:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0043] In a certain embodiment, the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment having the following feature:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0044] In an alternative embodiment, the anti-MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment having the following feature:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0045] In a certain embodiment, the anti-MUC1 antibody Fab fragment contained in the

pharmaceutical composition of the present invention is an anti-human MUC1 antibody Fab fragment having the following feature:

an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0046] The anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention binds to human cancer-specific MUC1. A method for measuring the binding activity of the obtained anti-human MUC1 antibody Fab fragment against human cancer-specific MUC1 includes methods such as ELISA and FACS. In the case of using, for example, ELISA, human cancer-specific MUC1-positive cells (e.g., T-47D cells) are immobilized onto an ELISA plate, to which the Fab fragment is then added and reacted, and then, an anti-Igk antibody or the like labeled with horseradish peroxidase or the like is reacted. Then, the binding of the secondary antibody is identified by activity measurement using a reagent for detecting the activity thereof (e.g., a chemiluminescent horseradish peroxidase substrate for the horseradish peroxidase label) or the like.

[0047] The anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention can be readily prepared by those skilled in the art using a method known in the art on the basis of sequence information on the heavy chain fragment and the light chain of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention disclosed herein. The anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention can be produced according to, but not particularly limited to, a method described in, for example, <4-4. Method for producing anti-human antibody Fab fragment> mentioned later.

[0048] 1-2. Labeling moiety

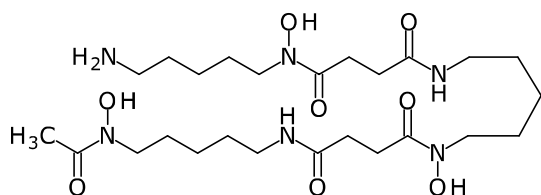
1-2-1. Labeling moiety comprising ligand

In a certain embodiment, the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a conjugate in which the labeling moiety is a ligand and a linker. In the present description, the "ligand" is a moiety capable of forming a chelate complex with a metal in the conjugate and means a group constituted by a chelating agent. The "constituted group" is a group having a bond by the removal of a proton from the chelating agent. The "chelating agent" is a compound that can form a coordinate bond with a metal.

[0049]

In a certain embodiment, examples of the chelating agent constituting the ligand include siderophore and non-siderophore when the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a ligand and a linker. Examples of a certain embodiment include MAG3 (mercaptoacetyl-glycyl-glycyl-glycine, CAS No: 66516-09-4) and known reactive derivatives thereof. Examples of the siderophore include hydroxamic acid type, catechol type, and mixed ligand type. Examples of the hydroxamic acid-type siderophore include ferrichrome, deferoxamine (DFO) represented by the following formula:

[Chemical Formula 4]



fusarinine C, ornibactin, rhodotorulic acid, and known reactive derivatives thereof.

Examples of the catechol-type siderophore include enterobactin, bacillibactin, vibriobactin, and known reactive derivatives thereof. Examples of the mixed ligand-type siderophore include azotobactin, pyoverdine, yersiniabactin, and known reactive derivatives thereof. In the case of the siderophore, DFO can be reacted via its reactive functional group $-NH_2$ with the linker or the Fab fragment, and the siderophore other than DFO can also be reacted via its reactive functional group such as a carboxy group, a hydroxy group, or an amino group with

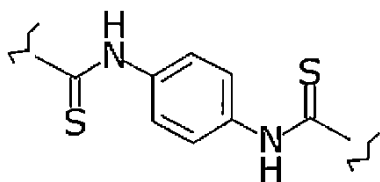
the linker or the Fab fragment by a method usually used by those skilled in the art.

[0050] Examples of the non-siderophore include DTPA (diethylenetriaminepentaacetic acid, CAS No: 67-43-6), DTPA-BMA (1,7-bis(methylcarbamoylmethyl)-1,4,7-triazaheptane-1,4,7-triacetic acid, CAS No: 119895-95-3), EOB-DTPA (ethoxybenzyl-DTPA, 2-[[[(2S)-2-[bis(carboxymethyl)amino]-3-(4-ethoxyphenyl)propyl]-[2-[bis(carboxymethyl)amino]ethyl]amino]acetic acid), TTHA (triethylenetetraminehexaacetic acid, CAS No: 869-52-3), DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, CAS No: 217973-03-0), HP-DO3A (10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, CAS No: 120041-08-9), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, CAS No: 60239-18-1), and known reactive derivatives thereof.

[0051] When the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a ligand and a linker, the "chelating agent" constituting the ligand is preferably DFO.

[0052] The "linker is a group that creates a distance between the anti-human antibody Fab fragment and the ligand. In a certain embodiment, when the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a ligand and a linker, examples of the linker that creates a distance between the anti-human antibody Fab fragment and the ligand include the following formula:

[Chemical Formula 5]



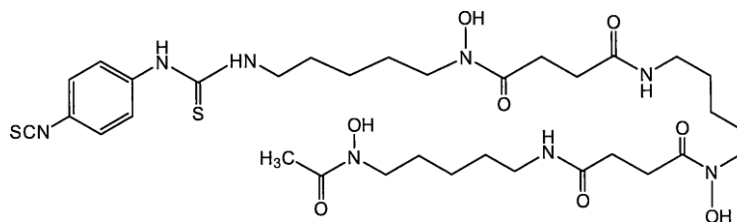
(hereinafter, designated as -C(=S)-NH-(1,4-phenylene)-NH-C(=S)-), -CH₂-(1,4-phenylene)-NH-C(=S)-, and -C(=O)-(C₁₋₂₀ alkylene)-C(=O)-. In this context, the "C₁₋₂₀ alkylene" is linear or branched alkylene having 1 to 20 carbon atoms. A certain embodiment of the C₁₋₂₀ alkylene is C₁₋₁₀ alkylene or C₁₋₂ alkylene. A certain embodiment of the C₁₋₂₀ alkylene is

ethylene. Examples of a reagent that can be used for forming the linker include HO-C(=O)-(C₁₋₂₀ alkylene)-C(=O)-OH, succinic acid, and p-phenylene diisothiocyanate.

[0053] When the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a ligand and a linker, the "linker" is preferably -C(=S)-NH-(1,4-phenylene)-NH-C(=S)-.

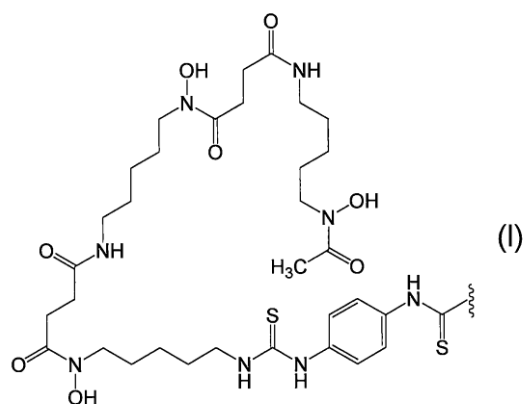
[0054] When the labeling moiety is a ligand and a linker, the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention may be produced by reacting the chelating agent forming the ligand with a substance obtained through the reaction of the anti-human antibody Fab fragment with the linker. The conjugate may also be produced by reacting the anti-human antibody Fab fragment with a substance obtained through the reaction of the linker with the chelating agent forming the ligand. As a reaction example, a substance obtained through the reaction of the amino group of the chelating agent with the linker is reacted with one or more amino groups (e.g., an N-terminal amino group and an amino group of a lysine side chain) of the anti-human antibody Fab fragment. When the labeling moiety is a ligand, it may be produced by reacting the chelating agent forming the ligand with the anti-human antibody Fab fragment. As a reaction example, the chelating agent is reacted with one or more amino groups (e.g., an N-terminal amino group and an amino group of a lysine side chain) of the anti-human antibody Fab fragment. Reaction of synthesizing thiourea by adding isothiocyanate to amine, reaction of synthesizing amide by adding carboxylic acid to amine, or the like can be used in the production of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention. The reaction can be performed by the application of a method known to those skilled in the art. A compound of the ligand bound to the linker in advance may be used as a starting material. Examples of the compound of the ligand bound to the linker include p-SCN-Bn-DFO (DFO substituted by a p-isothiocyanophenylaminothiocarbonyl group, CAS No: 1222468-90-7) represented by the following formula:

[Chemical Formula 6]



DTPA substituted by a p-isothiocabenzyl group (p-SCN-Bn-DTPA, CAS No: 102650-30-6), DOTA substituted by a p-isothiocabenzyl group (p-SCN-Bn-DOTA, CAS No: 127985-74-4), and p-SCN-Bn-CHX-A"-DTPA ([*(R)*]-2-amino-3-(4-isothiocabenzyl)propyl]-*trans*-(*S,S*)-cyclohexane-1,2-diamine-pentaacetic acid, CAS No: 157380-45-5).

[0055] In a certain embodiment, the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a conjugate in which the labeling moiety is a ligand and a linker represented by the following formula (I): [Chemical Formula 7]



wherein the wavy line represents binding to the anti-human antibody Fab fragment, where the anti-human antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human antibody Fab fragment.

[0056] As described above, when the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a ligand and a linker, the chelating agent constituting the ligand can form a chelating complex with a metal radioisotope. In the present description, the metal radioisotope is, for example, one that is used for a PET tracer etc., and examples thereof

include ^{89}Zr , ^{51}Mn , ^{52}Fe , ^{60}Cu , ^{67}Ga , ^{68}Ga , ^{72}As , $^{99\text{m}}\text{Tc}$, and ^{111}In . ^{89}Zr is preferable.

That is, the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention may be free of a metal radioisotope, or may contain ^{89}Zr as a metal radioisotope.

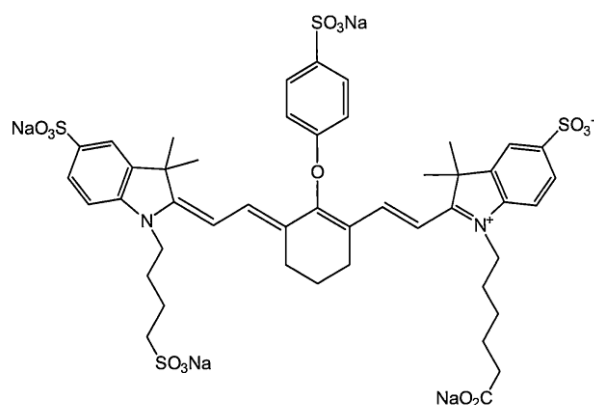
[0057] As for a form in which the pharmaceutical composition of the present invention is provided, it may be provided in a form free of a metal radioisotope, and labeled with the metal radioisotope immediately before use, or may be provided as a metal radioisotope-containing pharmaceutical composition used for diagnosis. For example, in the case of using a metal radioisotope with a short half-life (e.g. ^{89}Zr (half-life: 3.3 days)), it is preferable that the composition be provided in a form free of the metal radioisotope, and labeled with the metal radioisotope immediately before use.

[0058] 1-2-2. Labeling moiety comprising fluorescent dye

In a certain embodiment, when the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a fluorescent dye, a dye having absorption maximum and emission maximum at a near-infrared wavelength (650 to 1000 nm) usually used in photoimaging can be used as the fluorescent dye. Examples of a certain embodiment of the fluorescent dye include cyanine and indocyanine compounds. Examples of a certain embodiment include IRDye800CW (LI-COR, Inc.), Cy (Molecular Probes, Inc.), Alexa Fluor, BODIPY, and DyLight (Thermo Fisher Scientific Inc.), CF790 (Biotium, Inc.), DY (Dyomics GmbH), HiLyte Fluor 680 and HiLyte Fluor 750 (AnaSpec Inc.), and PULSAR650 and QUASAR670 (LGC Biosearch Technologies).

[0059] When the labeling moiety of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a fluorescent dye, the fluorescent dye is preferably IRDye800CW (LI-COR Biosciences) represented by the following formula:

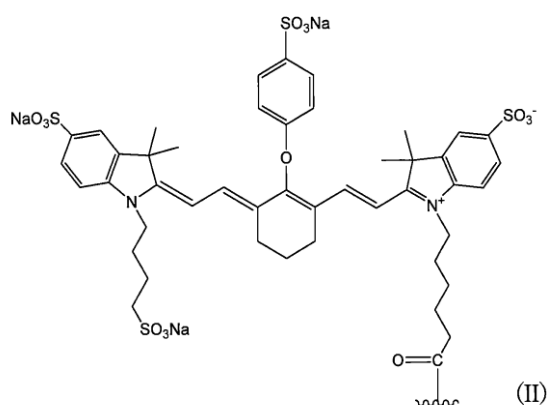
[Chemical Formula 8]



The fluorescent dye can be reacted via its carboxy group, hydroxy group, amino group, or the like or via an active group introduced by a method usually used by those skilled in the art with the anti-human antibody Fab fragment or the linker bound to the anti-human antibody Fab fragment. A certain embodiment of the fluorescent dye having an introduced active group is a fluorescent dye esterified with a N-hydroxysuccinimide (NHS) group. For example, NHS esters of IRDye800CW are commercially available, and they can be utilized.

[0060] In a certain embodiment, the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a conjugate in which the labeling moiety is a fluorescent dye represented by the following formula (II):

[Chemical Formula 9]



wherein the wavy line represents binding to the anti-human antibody Fab fragment, where the anti-human antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=O) group via an amino group in the anti-human antibody Fab fragment.

[0061] In the labeling moiety-anti-human antibody Fab fragment conjugate contained in the

pharmaceutical composition, the binding of the anti-human antibody Fab fragment to the labeling moiety can be appropriately performed by those skilled in the art using a known approach. For example, the labeling moiety can be bound to one or more amino groups (e.g., an N-terminal amino group and an amino group of an amino acid side chain), one or more thiol groups (e.g., a thiol group of an amino acid side chain), or one or more carboxyl groups (e.g., carboxyl groups of the C terminus and an amino acid side chain) of the anti-human antibody Fab fragment. A certain embodiment of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a conjugate in which the labeling moiety is bound to one or more amino groups of the anti-human antibody Fab fragment.

[0062] 1-3. Labeling moiety-anti-human antibody Fab fragment conjugate

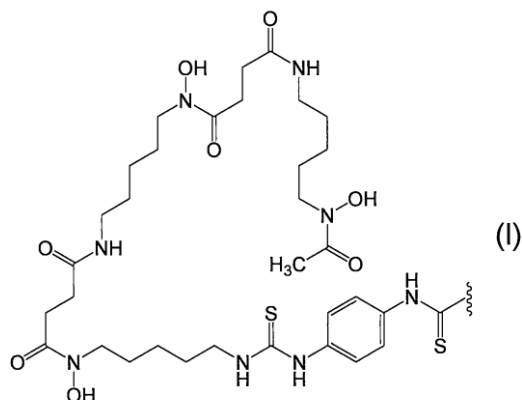
The pharmaceutical composition of the present invention comprises a labeling moiety-anti-human antibody Fab fragment conjugate. A certain embodiment of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate in which the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4, and

the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 10]



wherein the wavy line represents binding to the anti-human CEACAM5 antibody Fab fragment, where the anti-human CEACAM5 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human CEACAM5 antibody Fab fragment.

[0063] A certain embodiment of the anti-human CEACAM5 antibody Fab fragment contained in the labeling moiety-anti-human antibody Fab fragment conjugate is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0064] A certain embodiment of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a labeling moiety-anti-human MUC1 antibody Fab fragment conjugate in which the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a)

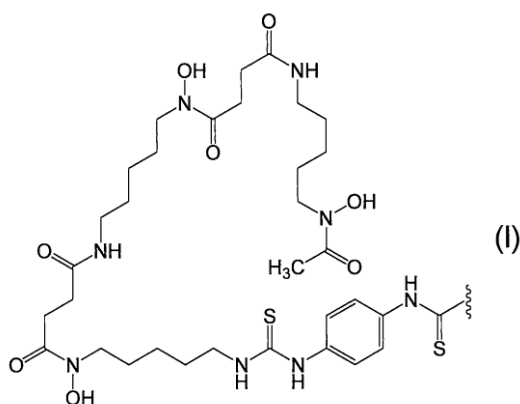
and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and

(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and

the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 11]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human MUC1 antibody Fab fragment.

[0065] A certain embodiment of the anti-human MUC1 antibody Fab fragment contained in the labeling moiety-anti-human antibody Fab fragment conjugate is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment

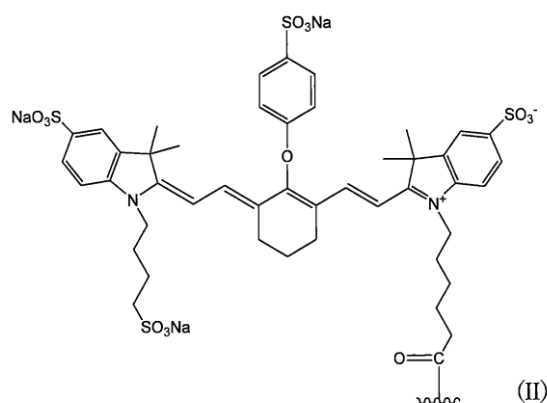
consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and
(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0066] A certain embodiment of the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a labeling moiety-anti-human MUC1 antibody Fab fragment conjugate in which the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and

(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and the labeling moiety is a group represented by the following formula (II):

[Chemical Formula 12]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=O) group via an amino group in the anti-human MUC1 antibody Fab fragment.

[0067] A certain embodiment of the anti-human MUC1 antibody Fab fragment contained in the labeling moiety-anti-human antibody Fab fragment conjugate is one or more selected from the group consisting of the following (a) and (b):

- (a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and
- (b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0068] The labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is a conjugate in which one or more labeling moieties are bound to the anti-human CEACAM5 antibody Fab fragment. A certain embodiment of the labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 25 labeling moieties. A

certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 23 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 16 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 11 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 10 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 1 to 9 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 4 to 23 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 4 to 16 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 4 to 10 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 4 to 9 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 3 to 23 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 3 to 16 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 3 to 10 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to 3 to 9 labeling moieties. A certain embodiment is the anti-human CEACAM5 antibody Fab fragment bound to at least one labeling moieties further comprising a metal.

[0069] The conjugate contained in the pharmaceutical composition of the present invention is a conjugate comprising one or more labeling moieties and an anti-human MUC1 antibody Fab fragment. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 27 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 23 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 15 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 11 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 9 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab

fragment bound to 1 to 7 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 5 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to 1 to 4 labeling moieties. A certain embodiment is the anti-human MUC1 antibody Fab fragment bound to at least one labeling moiety further comprising a metal.

[0070] The labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention encompasses free forms and salts thereof unless otherwise specified. In this context, the "salt thereof" is a salt that can be formed by the compound or the conjugate that may form an acid-addition salt or a salt with a base depending on the type of a substituent in the conjugate. Specific examples thereof include: acid-addition salts with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, nitric acid, and phosphoric acid, or organic acids such as formic acid, acetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, maleic acid, lactic acid, malic acid, mandelic acid, tartaric acid, dibenzoyltartaric acid, ditoluoyltartaric acid, citric acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, aspartic acid, and glutamic acid; salts with inorganic bases such as sodium, potassium, magnesium, calcium, and aluminum, or organic bases such as methylamine, ethylamine, ethanolamine, lysine, and ornithine; salts with various amino acids and amino acid derivatives, such as acetylleucine; and ammonium salts. For example, DFO exists as deferoxamine methanesulfonate or exists as other salts.

[0071] The concentration of the labeling moiety-anti-human antibody Fab fragment conjugate in the pharmaceutical composition of the present invention is not particularly limited as long as it is a concentration allowing diagnostically or therapeutically effective action to be exhibited, and the concentration is preferably 1 to 100 mg/mL, more preferably 5 to 20 mg/mL.

[0072] 1-4. Buffering agent

As a buffering agent in the pharmaceutical composition of the present invention, citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or

trishydroxymethyl aminomethane can be used from the viewpoint of maintaining a pH in a range as described below to suppress impacts on coordination efficiency of a metal radioisotope to a ligand. The concentration of the buffering agent in the pharmaceutical composition of the present invention differs depending on the type of the buffering agent and a target pH, and is preferably 10 to 30 mmol/L.

[0073] A certain embodiment of the buffering agent in the pharmaceutical composition of the present invention is citric acid, and the concentration thereof is 10 to 30 mmol/L, preferably 15 to 25 mmol/L. A certain embodiment of the buffering agent in the pharmaceutical composition of the present invention is phosphoric acid, and the concentration thereof is 10 to 30 mmol/L, preferably 15 to 25 mmol/L.

[0074] 1-5. Stabilizer

As a stabilizer in the pharmaceutical composition of the present invention, sucrose or glycerin can be used from the viewpoint of suppressing generation of multimers, acidic charge variants or insoluble subvisible particles due to heat stress, light stress, shaking stress, or the like during preservation, and also suppressing a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation. The concentration of the stabilizer in the pharmaceutical composition of the present invention differs depending on the type of a stabilizer used, and is preferably 5 to 30 w/v%.

[0075] A certain embodiment of the stabilizer in the pharmaceutical composition of the present invention is sucrose, and the concentration thereof is 5 to 30 w/v%, preferably 15 to 25 w/v%. A certain embodiment of the buffering agent in the pharmaceutical composition of the present invention is glycerin, and the concentration thereof is 5 to 30 w/v%, preferably 15 to 25 w/v%.

[0076] 1-6. Nonionic surfactant

In the pharmaceutical composition of the present invention, a nonionic surfactant can be used. As the nonionic surfactant in the pharmaceutical composition of the present invention, Polysorbate 80, Polysorbate 20, Polysorbate 60 or Poloxamer 188 can be used from the viewpoint of suppressing generation of insoluble subvisible particles, and also

suppressing a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation. The Polysorbate 80, which is also called polyoxyethylene (20) sorbitan oleic acid ester, is a polyoxyethylene ether of anhydrous sorbitol having some of hydroxyl groups esterified with oleic acid, and has a structure in which about 20 moles of ethylene oxide groups are ether-bonded to 1 mole of sorbitan monooleate. The concentration of the nonionic surfactant in the pharmaceutical composition of the present invention differs depending on the type thereof, and is preferably 0.02 to 0.2 w/v%.

[0077] A certain embodiment of the nonionic surfactant in the pharmaceutical composition of the present invention is Polysorbate 80, and the concentration thereof is 0.02 to 0.2 w/v%, preferably 0.04 to 0.1 w/v%.

[0078] 1-7. pH

In the pharmaceutical composition of the present invention, the pH is 6.5 to 7.5, more preferably 6.5 to 7.0, from the viewpoint of suppressing generation of multimers, acidic charge variants or insoluble subvisible particles due to heat stress, light stress, shaking stress, or the like during preservation, and also suppressing a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation.

[0079] A preferred form of the pharmaceutical composition of the present invention comprises the anti-human CEACAM5 antibody Fab fragment conjugate or anti-human MUC1 antibody Fab fragment mentioned above at a concentration of 5 to 20 mg/mL as a labeling moiety-anti-human antibody Fab fragment conjugate, citric acid at 15 to 25 mmol/L as a buffering agent, sucrose at 15 to 25 w/v% as a stabilizer, and Polysorbate 80 at 0.04 to 0.1 w/v% as a nonionic surfactant, and has a pH of 6.5 to 7.0.

[0080] 1-8. Pharmaceutical composition for use in diagnosis

In a certain embodiment, the present invention relates to a pharmaceutical composition for use in diagnosis comprising a labeling moiety-anti-human antibody Fab fragment conjugate. When the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention comprises a

ligand as a labeling moiety, coordination of a metal radioisotope (e.g. ^{89}Zr) makes the conjugate detectable. When the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention comprises a fluorescent dye as a labeling moiety, the conjugate is a detectable conjugate. These detectable conjugates can be utilized as an early diagnostic drug, a staging drug or an intraoperative diagnostic drug (particularly a diagnostic drug for cancer). The intraoperative diagnostic drug means a diagnostic drug capable of identifying a lesion site and examining the property thereof during an operation such as a surgical operation or an endoscopic operation. When the pharmaceutical composition for use in diagnosis according to the present invention is used as an intraoperative diagnostic drug, the pharmaceutical composition for use in diagnosis is administered to the patient, for example, 2 to 32 hours before the operation, a certain embodiment of the pharmaceutical composition is administered to the patient 6 to 24 hours before the operation, and another form of the pharmaceutical composition is administered to the patient 2 hours before the operation.

[0081] The early diagnostic drug means a diagnostic drug aimed at making a diagnosis when a condition is not found or during an early disease state. For example, for cancers, the early diagnostic drug means a diagnostic drug which is used when a condition is not found or during stage 0 or stage 1.

[0082] The staging drug means a diagnostic drug capable of examining the degree of progression of a condition. For example, for cancers, it means a diagnostic drug capable of examining the stage thereof.

[0083] When the pharmaceutical composition of the present invention comprises the labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate, it can be used for diagnosing cancers expressing human CEACAM5. When the pharmaceutical composition of the present invention comprises the labeling moiety-anti-human CEACAM5 antibody Fab fragment conjugate, a certain embodiment thereof is preferably used for the pharmaceutical composition for diagnosing colorectal cancer, breast cancer, lung cancer, thyroid gland cancer or a cancer resulting from the metastasis thereof, and in particular, use for diagnosing

colorectal cancer or a cancer resulting from the metastasis of colorectal cancer is preferable. The cancer resulting from the metastasis of colorectal cancer is not particularly limited, and examples thereof include metastatic liver cancer.

[0084] When the pharmaceutical composition of the present invention comprises the labeling moiety-anti-human MUC1 antibody Fab fragment conjugate, it can be used for diagnosing cancers expressing human MUC1. When the pharmaceutical composition of the present invention comprises the labeling moiety-anti-human MUC1 antibody Fab fragment conjugate, a certain embodiment thereof is preferably used for diagnosing breast cancer, lung cancer, colorectal cancer, bladder cancer, skin cancer, thyroid gland cancer, stomach cancer, pancreatic cancer, kidney cancer, ovary cancer or uterine cervical cancer, and in particular, use for diagnosing breast cancer or bladder cancer is preferable.

[0085] 1-9. Dosage form and additives of pharmaceutical composition

The dosage form of the pharmaceutical composition of the present invention is not particularly limited, and a certain embodiment thereof is a liquid formulation, a frozen formulation or a lyophilized formulation. The pharmaceutical composition of the present invention can be used as, for example, a parenteral agent such as an injection or an agent for drip infusion, and administration is preferably performed by intravenous injection, local intramuscular injection to a target, subcutaneous injection, or the like. The dose of the detectable labeling moiety-anti-human antibody Fab fragment conjugate in the pharmaceutical composition of the present invention differs depending on the age or body weight of a patient, the dosage form of a formulation used, or the binding titer of the Fab fragment, etc. For example, approximately 0.001 mg/kg to 100 mg/kg based on the mass of the Fab fragment can be used per unit body weight of a patient.

[0086] To the pharmaceutical composition of the present invention, medicinal additives such as a suspension agent, a solubilizer, a tonicity agent, a preservative, an adsorption inhibitor, an excipient, a soothing agent, a sulfur-containing reducing agent and an antioxidant or the like can be appropriately added if desired.

Examples of the suspension agent include methylcellulose, hydroxyethylcellulose,

gum arabic, powdered tragacanth, carboxymethylcellulose sodium, and polyoxyethylene sorbitan monolaurate or the like.

Examples of the solubilizer include polyoxyethylene hydrogenated castor oil, nicotinic amide, polyoxyethylene sorbitan monolaurate, macrogol, and castor oil fatty acid ethyl ester or the like.

Examples of the tonicity agent include sodium chloride, potassium chloride, calcium chloride or the like.

Examples of the preservative include methyl paraoxybenzoate, ethyl paraoxybenzoate, sorbic acid, phenol, cresol, chlorocresol, and benzyl alcohol or the like.

Examples of the adsorption inhibitor include human serum albumin, lecithin, dextran, ethylene oxide-propylene oxide copolymers, hydroxypropylcellulose, methylcellulose, polyoxyethylene hydrogenated castor oil, and polyethylene glycol or the like.

Examples of the excipient include xylitol or the like.

Examples of the soothing agent include inositol, and lidocaine or the like.

Examples of the sulfur-containing reducing agent include those having a sulfhydryl group, such as N-acetylcysteine, N-acetylhomocysteine, thioctic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, glutathione, and thioalkanoic acid having 1 to 7 carbon atoms.

Examples of the antioxidant include erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, α -tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbyl palmitate, L-ascorbyl stearate, sodium hydrogen sulfite, sodium sulfite, triamyl gallate, propyl gallate, and chelating agents such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate and sodium metaphosphate.

[0087] 2. Use of pharmaceutical composition for use in diagnosis and diagnosis method

The present invention relates to use of the labeling moiety-anti-human antibody Fab fragment conjugate for producing a pharmaceutical composition for use in early diagnosis of a cancer, a pharmaceutical composition for use in staging or a pharmaceutical composition

for use in intraoperative diagnosis. In a certain embodiment, the present invention provides a pharmaceutical composition comprising the labeling moiety-anti-human antibody Fab fragment conjugate, for use in early diagnosis, staging or intraoperative diagnosis of a cancer.

[0088] The present invention also relates to a method for diagnosing a cancer, comprising preoperatively or intraoperatively administering the pharmaceutical composition comprising the labeling moiety-anti-human antibody Fab fragment conjugate to a subject. In this context, the "subject" is a human or any of other mammals in need of receiving the diagnosis. A certain embodiment is a human in need of receiving the diagnosis. The effective amount of the pharmaceutical composition of the present invention comprising the labeling moiety-anti-human antibody Fab fragment conjugate in the diagnosis method differs depending on the age or body weight of a patient, the dosage form of a formulation used, or the binding titer of the Fab fragment, etc. For example, approximately 0.001 mg/kg to 100 mg/kg based on the mass of the Fab fragment may be used per unit body weight of a patient. In the diagnosis method, administration of the pharmaceutical composition of the present invention comprising the labeling moiety-anti-human antibody Fab fragment conjugate is preferably performed by local intramuscular injection to a target tissue, subcutaneous injection, or the like. In the case of preoperatively administering the pharmaceutical composition of the present invention in the diagnosis method, the conjugate is administered to the patient, for example, 2 to 48 hours before the operation, a certain embodiment of the conjugate is administered to the patient 6 to 24 hours before the operation, and another form of the conjugate is administered to the patient 2 hours before the operation.

[0089] In another embodiment, the present invention also relates to use of the labeling moiety-anti-human antibody Fab fragment conjugate for producing the pharmaceutical composition of the present invention.

[0090] 3. Method for producing pharmaceutical composition comprising labeling moiety-anti-human antibody Fab fragment conjugate and method for stably preserving labeling moiety-anti-human antibody Fab fragment conjugate

In a certain embodiment, the present invention relates to a method for producing a

pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate. Specifically, in a certain embodiment, the present invention provides a method for producing a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of (a) producing and adding the labeling moiety-anti-human antibody Fab fragment conjugate; (b) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent; (c) adding sucrose or glycerin as a stabilizer; (d) adding Polysorbate 80 as a nonionic surfactant; and (e) adjusting the pH to 6.5 to 7.5. The order of the addition is not particularly limited.

[0091] In a certain embodiment, the present invention relates to a method for stably preserving the labeling moiety-anti-human antibody Fab fragment conjugate. In the present description, the term "stably preserving" refers to suppression of generation of multimers or insoluble subvisible particles during preservation of the labeling moiety-anti-human antibody Fab fragment conjugate. In a certain embodiment, the term "stably preserving" as used herein represents a concept including suppression of a decrease in coordination efficiency of a metal radioisotope to a ligand and fluorescent dye color degradation. Specifically, in a certain embodiment, the present invention provides a method for stably preserving a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of: (a) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent to a solution containing the labeling moiety-anti-human antibody Fab fragment conjugate; (b) adding sucrose or glycerin as a stabilizer to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate; (c) adding Polysorbate 80 as a nonionic surfactant to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate; and (d) adjusting the pH of the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate to 6.5 to 7.5. The order of the addition is not particularly limited.

[0092] For the production method and the method for stably preserving, the structures of the anti-human antibody Fab fragment and the labeling moiety, the structure of the labeling

moiety-anti-human antibody Fab fragment conjugate, and the like are as described in the section "1. Pharmaceutical composition". The ranges of the concentrations, the ranges of the pHs, and the like of the buffering agent, the stabilizer and the nonionic surfactant are also as described in the section "1. Pharmaceutical composition". The steps may be carried out in any order.

[0093] When the labeling moiety-anti-human antibody Fab fragment conjugate comprises a ligand as the labeling moiety in the production method and the method, a certain embodiment may comprise the step of coordinating a metal radioisotope. The type of the metal radioisotope, and the like are as described in the section "1. Pharmaceutical composition".

[0094] Further, in the production method and the method, a certain embodiment may comprise the step of performing freezing or the step of performing lyophilization. As the freezing method or the lyophilization method, a known method can be used.

[0095] 4. Method for producing labeling moiety-anti-human antibody Fab fragment conjugate

4-1. Polynucleotide encoding anti-human antibody Fab fragment

When the conjugate contained in the pharmaceutical composition of the present invention comprises the anti-human CEACAM5 antibody Fab fragment, a certain embodiment of a polynucleotide encoding the anti-human antibody Fab fragment comprises a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment, and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment.

[0096] In a certain embodiment, the polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2, or a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid

positions 1 to 112 of SEQ ID NO: 4.

[0097] Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2 include a polynucleotide comprising a nucleotide sequence from nucleotide positions 1 to 363 of SEQ ID NO: 1. Examples of the polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4 include a polynucleotide comprising a nucleotide sequence from nucleotide positions 1 to 336 of SEQ ID NO: 3.

[0098] In a preferred embodiment, the polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2, or a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0099] Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 1. Examples of the polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 3.

[0100] When the conjugate contained in the pharmaceutical composition of the present invention comprises the anti-human MUC1 antibody Fab fragment, a certain embodiment of a polynucleotide encoding the anti-human antibody Fab fragment comprises a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment of the anti-human MUC1 antibody Fab fragment, and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment.

[0101] A certain embodiment of the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence

encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12, or a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 14.

[0102] Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 11. Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 14 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 13.

[0103] In a preferred embodiment, the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6, or a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 8.

[0104] Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 5.

Examples of the polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 8 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 7.

[0105] In a certain embodiment, the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0106] Examples of the polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence

represented by SEQ ID NO: 16 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 15.

[0107] In a preferred embodiment, the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0108] Examples of the polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10 include a polynucleotide comprising the nucleotide sequence represented by SEQ ID NO: 9.

[0109] The polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention can be synthesized through the use of a gene synthesis method known in the art on the basis of nucleotide sequences designed from the amino acid sequences of the heavy chain fragment and the light chain of the anti-human CEACAM5 antibody Fab fragment or the anti-human MUC1 antibody Fab fragment. Various methods known to those skilled in the art, such as methods for synthesizing an antibody gene described in International Publication No. WO 90/07861 can be used as such gene synthesis methods.

[0110] 4-2. Expression Vector of polynucleotide encoding anti-human antibody Fab fragment

The expression vector of the polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment in the conjugate contained in the pharmaceutical composition of the present invention includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment, an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment.

[0111] The preferred expression vector includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2, an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 112 of SEQ ID NO: 4, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2 and a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 112 of SEQ ID NO: 4.

[0112] The more preferred expression vector encoding the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2, an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 and a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0113] The expression vector of the polynucleotide encoding the anti-human MUC1 antibody Fab fragment in the conjugate contained in the pharmaceutical composition of the present invention includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human MUC1 antibody Fab fragment, an expression vector comprising a polynucleotide comprising

a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human MUC1 antibody Fab fragment and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment.

[0114] The preferred expression vector includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14, an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a polynucleotide comprising a nucleotide sequence encoding a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0115] The more preferred expression vector includes an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8, an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10, and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0116] These expression vectors are not particularly limited as long as a polypeptide encoded by the polynucleotide can be produced in various host cells of prokaryotic cells and/or eukaryotic cells. Examples of such expression vectors include plasmid vectors and virus vectors (e.g., adenovirus and retrovirus) or the like. Preferably, pEE6.4 or

pEE12.4 (Lonza Group AG) can be used.

[0117] These expression vectors may comprise a promoter operably linked to a gene encoding the heavy chain fragment and/or the light chain in the polynucleotide encoding the anti-human antibody Fab fragment contained in the pharmaceutical composition of the present invention. Examples of the promoter for expressing the Fab fragment contained in the pharmaceutical composition of the present invention in a host cell include Trp promoter, lac promoter, recA promoter, λ PL promoter, lpp promoter, and tac promoter or the like, when the host cell is a bacterium of the genus *Escherichia*. Examples of the promoter for expression in yeasts include PH05 promoter, PGK promoter, GAP promoter, and ADH promoter or the like. Examples of the promoter for expression in bacteria of the genus *Bacillus* include SL01 promoter, SP02 promoter, and penP promoter or the like. Examples thereof include promoters derived from viruses such as CMV, RSV, and SV40, retrovirus promoter, actin promoter, EF (elongation factor) 1 α promoter, and heat shock promoter or the like, when the host is a eukaryotic cell such as a mammalian cell.

[0118] In the case of using a bacterium, particularly, *E. coli*, as a host cell, these expression vector may further comprise a start codon, a stop codon, a terminator region and a replicable unit. On the other hand, in the case of using a yeast, an animal cell or an insect cell as a host, the expression vector encoding the anti-human antibody Fab fragment contained in the pharmaceutical composition of the present invention may comprise a start codon and a stop codon. In this case, an enhancer sequence, 5' and 3' untranslated regions of a gene encoding the heavy chain fragment and/or the light chain contained in the pharmaceutical composition of the present invention, a secretion signal sequence, a splicing junction, a polyadenylation site, or a replicable unit, etc. may be contained therein. Also, a selective marker usually used (e.g., tetracycline resistance gene, ampicillin resistance gene, kanamycin resistance gene, neomycin resistance gene, and dihydrofolate reductase gene) may be contained therein according to a purpose.

[0119] 4-3. Host cell transformed with expression vector

The host cell transformed with the expression vector of the polynucleotide encoding

the anti-human CEACAM5 antibody Fab fragment includes a host cell transformed with the expression vector selected from the group consisting of the following (a) to (d):

- (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention;
- (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention;
- (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention; and
- (d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human CEACAM5 antibody Fab fragment contained in the pharmaceutical composition of the present invention.

[0120] In one embodiment, the host cell transformed with the expression vector of the polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment is a host cell selected from the group consisting of the following (a) to (d):

- (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 112 of SEQ ID NO: 4;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2 and a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 112 of SEQ ID NO: 4; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 121 of SEQ ID NO: 2 and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by amino acid positions 1 to 112 of SEQ ID NO: 4.

[0121] In one embodiment, the host cell transformed with the expression vector of the polynucleotide encoding the anti-human CEACAM5 antibody Fab fragment is a host cell selected from the group consisting of the following (a) to (d):

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid

sequence represented by SEQ ID NO: 2 and a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 and expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

[0122] The host cell transformed with the expression vector of the polynucleotide encoding the anti-human MUC1 antibody Fab fragment includes a host cell transformed with the expression vector selected from the group consisting of the following (a) to (d):

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention and a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the heavy chain fragment of the anti-human MUC1 antibody Fab fragment contained in the pharmaceutical composition of the present invention and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding the light chain of the anti-human MUC1 antibody Fab fragment contained in the

pharmaceutical composition of the present invention.

[0123] In one embodiment, the host cell transformed with the expression vector of the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a host cell selected from the group consisting of the following (a) to (d):

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16.

[0124] In one embodiment, the host cell transformed with the expression vector of the polynucleotide encoding the anti-human MUC1 antibody Fab fragment is a host cell selected from the group consisting of the following (a) to (d):

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid

sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and expression vector comprising a polynucleotide comprising a nucleotide sequence encoding a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

[0125] The host cell to be transformed is not particularly limited as long as it is compatible with the expression vector used and can be transformed with the expression vector to express the Fab fragment. Examples thereof include various cells such as natural cells and artificially established cells usually used in the technical field of the present invention (e.g., bacteria (bacteria of the genus *Escherichia* and bacteria of the genus *Bacillus*), yeasts (the genus *Saccharomyces*, the genus *Pichia*, etc.), animal cells and insect cells (e.g., Sf9), etc.), and mammalian cell lines (e.g., cultured cells such as CHOK1SV cells, CHO-DG44 cells, and 293 cells, etc.). The transformation itself can be performed by a known method, for example, a calcium phosphate method or an electroporation method.

[0126] 4-4. Method for producing anti-human antibody Fab fragment

The method for producing the anti-human antibody Fab fragment, preferably the method for producing the anti-human CEACAM5 antibody Fab fragment or the anti-human MUC1 antibody Fab fragment, comprises the step of culturing the transformed host cell to express the anti-human antibody Fab fragment.

[0127] In the method for producing an anti-human antibody Fab fragment, the transformed host cell can be cultured in a nutrient medium. The nutrient medium preferably contains a nutrient source such as a carbon source, an inorganic nitrogen source or an organic nitrogen source necessary for the growth of the transformed host cell. Examples of the carbon source include glucose, dextran, soluble starch, and sucrose or the like. Examples of the inorganic nitrogen source or the organic nitrogen source include ammonium salts, nitrates, amino acids, corn steep liquor, peptone, casein, meat extracts, soymeal, and potato extracts or the like. Also, other nutrients (e.g., inorganic salts (e.g., calcium chloride, sodium dihydrogen phosphate, and magnesium chloride), vitamins, and antibiotics (e.g., tetracycline, neomycin, ampicillin, and kanamycin, etc.), etc.) may be contained therein, if desired.

[0128] The culture itself of the transformed host cell is performed by a known method. Culture conditions, for example, temperature, medium pH and culture time, are appropriately selected. When the host is, for example, an animal cell, MEM medium (Science; 1952; 122: 501), DMEM medium (Virology; 1959; 8: 396-97), RPMI1640 medium (J. Am. Med. Assoc.; 1967; 199: 519-24), 199 medium (Proc. Soc. Exp. Biol. Med.; 1950; 73:1-8), or the like containing approximately 5 to 20% fetal bovine serum can be used as a medium. The medium pH is preferably approximately 6 to 8. The culture is usually performed at approximately 30 to 40°C for approximately 15 to 336 hours, and aeration or stirring can also be performed, if necessary. When the host is an insect cell, examples thereof include Grace's medium (PNAS; 1985; 82: 8404-8) or the like containing fetal bovine serum. Its pH is preferably approximately 5 to 8. The culture is usually performed at approximately 20 to 40°C for 15 to 100 hours, and aeration or stirring can also be performed, if necessary. When the host is a bacterium, an actinomycete, a yeast, or a filamentous fungus, for example, a liquid medium containing the nutrient source described above is appropriate. A medium of pH 5 to 8 is preferred. When the host is *E. coli*, preferred examples of the medium include LB medium and M9 medium (Miller et al., Exp. Mol. Genet, Cold Spring Harbor Laboratory; 1972: 431) or the like. In such a case, the culture can usually be performed at 14 to 43°C for approximately 3 to 24 hours with aeration or stirring, if necessary. When the

host is a bacterium of the genus *Bacillus*, it can usually be performed at 30 to 40°C for approximately 16 to 96 hours with aeration or stirring, if necessary. When the host is a yeast, examples of the medium include Burkholder minimum medium (PNAS; 1980; 77: 4505-8). Its pH is desirably 5 to 8. The culture is usually performed at approximately 20 to 35°C for approximately 14 to 144 hours, and aeration or stirring can also be performed, if necessary.

[0129] The method for producing an anti-human antibody Fab fragment can comprise the step of recovering, preferably isolating or purifying, the expressed anti-human antibody Fab fragment, in addition to the step of culturing the transformed host cell to express the anti-human antibody Fab fragment. Examples of the isolation or purification method include: methods exploiting solubility, such as salting out and a solvent precipitation method or the like; methods exploiting difference in molecular weight, such as dialysis, ultrafiltration, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis or the like; methods exploiting charge, such as ion-exchange chromatography and hydroxylapatite chromatography or the like; methods exploiting specific affinity, such as affinity chromatography or the like; methods exploiting difference in hydrophobicity, such as reverse-phase high-performance liquid chromatography or the like; and methods exploiting difference in isoelectric point, such as isoelectric focusing or the like; or the like.

[0130] 4-5. Method for producing labeling moiety-anti-human antibody Fab fragment conjugate

The method for producing the labeling moiety-anti-human antibody Fab fragment conjugate contained in the pharmaceutical composition of the present invention comprises the step of covalently binding the anti-human antibody Fab fragment to a labeling moiety. The method for producing a conjugate may also comprise the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; and covalently binding the Fab fragment to a labeling moiety. The method for producing a conjugate may also comprise the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; recovering the expressed Fab fragment; and covalently binding the

Fab fragment to a labeling moiety. The linker, ligand, or fluorescent dye, etc. used can employ those described in the section "1. Pharmaceutical composition".

[0131] A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; and binding the Fab fragment via a linker to a ligand. A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; recovering the expressed Fab fragment; and binding the Fab fragment via a linker to a ligand.

[0132] A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; and i) binding the Fab fragment via a linker to a ligand or ii) covalently binding the Fab fragment directly to a ligand. A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment, recovering the expressed Fab fragment; and i) binding the Fab fragment via a linker to a ligand or ii) covalently binding the Fab fragment directly to a ligand.

[0133] A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment; and i) binding the Fab fragment via a linker to a fluorescent dye or ii) covalently binding the Fab fragment directly to a fluorescent dye. A certain embodiment of the method for producing a conjugate is a method comprising the steps of: culturing the transformed host cell to express the anti-human antibody Fab fragment, recovering the expressed Fab fragment; and i) binding the Fab fragment via a linker to a fluorescent dye or ii) covalently binding the Fab fragment directly to a fluorescent dye.

[0134] The present invention is generally described above. Particular Examples will be provided here for reference in order to obtain further understanding. However, these are given for illustrative purposes and do not limit the present invention.

EXAMPLES

[0135] Experiment 1-1: Preparation of anti-human CEACAM5 antibody Fab fragment

An antibody having variable regions expected not to attenuate affinity even by the binding of a labeling moiety was designed using a molecular model of a humanized antibody constructed in accordance with the literature (Proteins: Structure, Function, and Bioinformatics; 2014; 82: 1624-1635) after humanization of mouse-derived anti-human CEACAM5 antibody T84.66 with reference to the method described in the literature (Protein Eng. Des. Sel.; 2004; 17: 481-489).

[0136] A gene encoding a signal sequence (MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 17)) was connected to the 5' side of the heavy chain fragment gene (SEQ ID NO: 1) of the antibody, and this heavy chain fragment gene was inserted to GS vector pEE6.4 (Lonza Group AG). Also, a gene encoding a signal sequence (MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 18)) was connected to the 5' side of the light chain gene (SEQ ID NO: 3) of the antibody, and the light chain gene was inserted to GS vector pEE12.4 (Lonza Group AG). The aforementioned pEE vectors respectively having inserts of the heavy chain fragment and light chain genes of the antibody were cleaved with restriction enzymes NotI and PvuI and ligated using ligation kit TAKARA Ligation Kit Ver 2.1 (Takara Bio Inc.) to construct a GS vector having both the inserts of the heavy chain fragment and light chain genes.

[0137] The antibody was expressed by two types of methods, transient expression and constitutive expression, using the aforementioned GS vector having both the inserts of the heavy chain fragment and light chain genes. For the transient expression, Expi293F cells (Thermo Fisher Scientific Inc.) cultured into approximately 3000000 cells/mL in Expi293 Expression Medium (Thermo Fisher Scientific Inc.) were transfected with the aforementioned GS vector having both the inserts of the heavy chain fragment and light chain genes using ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific Inc.) and cultured for 5 to 7 days. The culture supernatant was purified using KappaSelect (GE Healthcare Japan Corp.) to obtain a Fab fragment. For the constitutive expression, CHOK1SV cells (Lonza Group AG) were transfected with a linear vector obtained with PvuI from the

aforementioned GS vector having both the inserts of the heavy chain fragment and light chain genes, by electroporation using Gene Pulser (Bio-Rad Laboratories, Inc.). On the day following the transfection, methionine sulfoximine was added thereto, followed by culture for 5 to 7 days. The cells were inoculated to a semisolid medium containing methylcellulose. After colony formation, cells having a large expression level of the Fab fragment were obtained using ClonePix FL (Molecular Devices, LLC). The culture supernatant of the cells was purified using Capto L (GE Healthcare Japan Corp.), Q Sepharose Fast Flow (GE Healthcare Japan Corp.), and BioPro S75 (YMC Co., Ltd.) to obtain a Fab fragment.

[0138] The nucleotide sequence encoding the heavy chain fragment of the prepared anti-human CEACAM5 antibody Fab fragment (designated as PB009-1) is shown in SEQ ID NO: 1, and the amino acid sequence encoded thereby is shown in SEQ ID NO: 2. The nucleotide sequence encoding the light chain of PB009-1 is shown in SEQ ID NO: 3, and the amino acid sequence encoded thereby is shown in SEQ ID NO: 4. The heavy chain variable region of PB009-1 consists of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2, and heavy chain CDR1, CDR2, and CDR3 consist of amino acid sequences from amino acid positions 31 to 35, 50 to 66, and 99 to 110, respectively, of SEQ ID NO: 2. The light chain variable region of PB009-1 consists of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4, and light chain CDR1, CDR2, and CDR3 consist of amino acid sequences from amino acid positions 24 to 38, 54 to 60, and 93 to 101, respectively, of SEQ ID NO: 4.

[0139] The variable regions and the CDR sequences were determined according to the Kabat numbering (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institute of Health, Bethesda).

[0140] Experiment 1-2: Labeling of chelating agent of anti-human CEACAM5 antibody Fab fragment

p-SCN-Bn-DFO (DFO substituted by a p-isothiocyanophenylaminothiocarbonyl group) (Macrocyclics, Inc.) was used in the binding of chelating agent DFO to anti-human

CEACAM5 antibody Fab fragment PB009-1. A 1/5 amount of a 0.1 M sodium carbonate solution (pH 9.0) was added to a Fab fragment solution adjusted to 1 mg/mL with phosphate-buffered saline (pH 7.4). p-SCN-Bn-DFO was added thereto at a final concentration of 1 mg/mL and reacted at 37°C for 1.5 hours. After the reaction, a DFO-anti-human CEACAM5 antibody Fab fragment conjugate bound to DFO via a linker (-C(=S)-NH-(1,4-phenylene)-NH-C(=S)-) (designated as PB009-2) was purified using Amicon Ultra 3K-0.5 mL centrifugal filter (Merck Millipore).

[0141] The number of ligands constituted by DFO bound to PB009-2 was confirmed by mass spectrometry. PB009-2 was desalted using MassPREP Micro Desalting Column (Waters Corp.), and measurement was carried out using SYNAPT G2 mass spectrometer (Waters Corp.). As a result, a molecule in which at least 3 to 10 ligands constituted by DFO were bound to one PB009-1 was confirmed.

[0142] Experiment 1-3: Examination of effect of pH on stabilization of DFO-anti-human CEACAM5 antibody Fab fragment conjugate (effect on multimer generation and acidic-side charge analog generation)

For liquid formulation comprising PB009-2, an effect of pH on stabilization of PB009-2 was evaluated. In this test, samples A-1 to A-6 shown in Table 1 were prepared by adding a buffering agent and a nonionic surfactant to the liquid formulation comprising PB009-2 in such a manner that the final concentration of PB009-2 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0143] [Table 1]

Table 1

Sample No.	Buffering agent	pH	Nonionic surfactant
A-1	20mmol/L Citric acid	5.0	0.1w/v% Polysorbate 80
A-2		6.0	
A-3		7.0	
A-4	20mmol/L Tris (trishydroxymethylaminomethane)	7.0	
A-5		7.5	
A-6		8.0	

[0144] For evaluating the stability of the liquid formulation, a heat stability test was conducted on each sample in a normally placed state. In the heat stability test, the stability of PB009-2 after storage at 25°C for 1 week was evaluated on the basis of the amount of multimers measured by the size exclusion chromatography method (SE-HPLC method) and the amount of acidic charge variants measured by the imaging capillary isoelectric focusing method (icIEF method). Analysis conditions are as follows.

[0145] [Size exclusion chromatography method (SE-HPLC method)]

In SE-HPLC measurement, G3000SWXL (TOSOH CORPORATION) was connected to a HPLC system, and a mobile phase having a composition of phosphoric acid at 20 mmol/L and sodium chloride at 400 mmol/L (pH 7.0) was fed at a flow rate of 0.5 mL/min. The injection amount of the sample was 50 µg in terms of PB009-2 (e.g. 10 µL in the case of 5 mg/mL). The column temperature was set to 30°C, the sample temperature was set to 5°C, and detection was performed at UV 280 nm.

[0146] [Imaging capillary isoelectric focusing method (icIEF method)]

In icIEF measurement, cIEF cartridge (Protein Simple, Inc.) was connected to iCE3 system, and the measurement was performed. 188 µL of a sample matrix consisting of urea, methylcellulose, Pharmalyte 3-10, pI marker 5.12 and pI marker 9.77 was mixed with 12 µL of a sample diluted to a PB009-2 concentration of 5 mg/mL with ultrapure water to give a measurement sample. Prefocusing was performed at 1500 V for 1 minute, and Focusing was performed at 3000 V for 6 minutes.

[0147] After the SE-HPLC measurement, the area of detected multimers were measured by an automatic analysis method to determine the amount of multimers (%). The amount of multimers (%) was defined in terms of a percentage (%) by measuring the total area of multimer peaks detected by the SE-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. Here, the main peak refers to a peak of an active main body (PB009-2 which is not multimerized).

[0148] The area of acidic charge variants detected by the icIEF method was measured by the automatic analysis method to determine the amount of acidic charge variants (%). The amount of acidic charge variants (%) is defined in terms of a percentage (%) by measuring the total area of acidic-side charge analog peaks detected by the icIEF method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. Here, the main peak refers to a peak of an active main body (PB009-2 which is not variants).

[0149] Table 2 shows the evaluation results obtained by the SE-HPLC method and the icIEF method in this experiment. The results show that the amount of multimers in SE-HPLC (%) after storage at 25°C for 1 week tended to increase with decrease in pH, and became minimum at a pH of about 7.0. The amount of acidic charge variants (%) after storage at 25°C for 1 week tended to decrease with decrease in pH. Comprehensive judgement on the above results revealed that the optimum pH of the liquid formulation comprising PB009-2 was about 7.0, from the viewpoint of stability.

[0150] [Table 2]

Table 2

Sample No.	Buffering agent	pH	Amount of multimers in SE-HPLC (%)		Amount of acidic-side charge analogs in icIEF (%)	
			At start of test	After storage at 25°C for 1 week	At start of test	After storage at 25°C for 1 week
A-1	20mmol/L Citric acid	5.0	2.6	12.3	46.2	37.6
A-2		6.0	2.5	6.9	42.0	41.6
A-3		7.0	2.6	5.2	45.2	45.6
A-4	20mmol/L Tris	7.0	2.3	4.8	42.8	44.5
A-5		7.5	2.3	5.1	45.4	42.6
A-6		8.0	2.4	5.9	45.0	45.6

[0151] Experiment 1-4: Examination of effects of stabilizer and nonionic surfactant on stabilization of DFO-anti-human CEACAM5 antibody Fab fragment conjugate (effects on multimer generation and acidic-side charge analog generation)

For liquid formulation comprising PB009-2, effects of various stabilizers or nonionic surfactants on stabilization of PB009-2 were evaluated. In this test, samples B-1 to B-10 shown in Table 3 were prepared by adding a buffering agent and an additive to the liquid formulation comprising PB009-2 in such a manner that the final concentration of PB009-2 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0152] [Table 3]

Table 3

Sample No.	Buffering agent	pH	Additive
B-1	20mmol/L Tris	7.0	0.1w/v% Polysorbate 80
B-2			140mM Arginine, 0.1w/v% Polysorbate 80
B-3			140mM Histidine, 0.1w/v% Polysorbate 80
B-4			140mM Aspartic acid, 0.1w/v% Polysorbate 80
B-5			280mM Glycine, 0.1w/v% Polysorbate 80
B-6			280mmol/L Sucrose, 0.1w/v% Polysorbate 80
B-7			280mmol/L Sorbitol, 0.1w/v% Polysorbate 80
B-8			140mmol/L Sodium acetate, 0.1w/v% Polysorbate 80
B-9			140mmol/L Sodium chloride, 0.1w/v% Polysorbate 80
B-10			20w/v% Glycerin, 0.1w/v% Polysorbate 80

[0153] For evaluating the stability of the liquid formulation, a heat stability test was conducted on each sample in a normally placed state. In the heat stability test, the stability of PB009-2 after storage at 25°C for 1 week was evaluated on the basis of the amount of multimers measured by the SE-HPLC method and the amount of acidic charge variants measured by the icIEF method. The experimental procedure of the SE-HPLC method is as follows.

[0154] [Size exclusion chromatography method (SE-HPLC method)]

In SE-HPLC measurement, AdvanceBio SEC 300A column (Agilent Technologies) was connected to a HPLC system, and a mobile phase having a composition of phosphoric

acid at 20 mmol/L and sodium chloride at 400 mmol/L (pH 7.0) was fed at a flow rate of 0.5 mL/min. The injection amount of the sample was 50 µg in terms of PB009-2 (e.g. 10 µL in the case of 5 mg/mL). The column temperature was set to 30°C, the sample temperature was set to 5°C, and detection was performed at UV 280 nm.

[0155] After the SE-HPLC measurement, the area of detected multimers were measured by an automatic analysis method to determine the amount of multimers (%). The amount of multimers (%) was defined in terms of a percentage (%) by measuring the total area of multimer peaks detected by the SE-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. Here, the main peak refers to a peak of an active main body (PB009-2 which is not multimerized).

[0156] The analysis conditions of the icIEF method are the same as those in Experiment 1-3.

[0157] Table 4 shows the evaluation results obtained by the SE-HPLC method and the icIEF method in this experiment. First, an increase in the amount of multimers in SE-HPLC (%) after storage at 25°C for 1 week tended to be suppressed in samples containing histidine, sucrose and glycerin, respectively. The amount of acidic charge variants (%) after storage at 25°C for 1 week increased in samples containing histidine and aspartic acid, respectively. Comprehensive judgement on the above results revealed that sucrose or glycerin was desirable as a stabilizer for the liquid formulation comprising PB009-2, from the viewpoint of stability.

[0158] [Table 4]

Table 4

Sample No.	Amount of multimers in SE-HPLC (%)		Amount of acidic-side charge analogs in icIEF (%)	
	At start of test	After storage at 25°C for 1 week	At start of test	After storage at 25°C for 1 week
B-1	1.4	4.0	44.0	45.7
B-2	1.4	3.6	42.7	46.2
B-3	1.4	3.1	41.4	53.3
B-4	1.3	3.7	48.5	54.9
B-5	1.5	4.0	40.2	44.8
B-6	1.4	3.1	42.1	46.1
B-7	1.4	3.4	44.8	46.1
B-8	1.4	3.4	39.4	46.0
B-9	1.6	4.0	42.7	44.4
B-10	1.2	2.6	40.4	43.0

[0159] Experiment 1-5: Examination of effect of surfactant on stabilization of DFO-anti human CEACAM5 antibody Fab fragment conjugate (effect on insoluble subvisible particle generation)

As liquid formulation formulated to comprise PB009-2 at 10 mg/mL and citric acid at 20 mmol/L and have a pH of 7.0, samples comprising Polysorbate 80 as a surfactant at 0 to 0.6 w/v% were prepared (samples No. C-1 to C-7: see Table 5). The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0160] [Table 5]

Table 5

Sample No.	Buffering agent	pH	Nonionic surfactant
C-1	20mmol/L Citric acid	7.0	0w/v% Polysorbate 80
C-2			0.02w/v% Polysorbate 80
C-3			0.05w/v% Polysorbate 80
C-4			0.1w/v% Polysorbate 80
C-5			0.2w/v% Polysorbate 80
C-6			0.4w/v% Polysorbate 80
C-7			0.6w/v% Polysorbate 80

[0161] For each sample, the number of insoluble subvisible particles after shaking and after freezing and thawing was measured using a light obscuration particle count method. The shaking test was conducted by shaking the sample at 150 rpm for 24 hours. The freezing and thawing test was conducted by carrying out a total of three processes each comprising freezing the sample at -80°C for 4 hours or more, and then thawing the sample at 5°C for 4 hours or more. The analysis conditions of the light obscuration particle count method are as follows.

[0162] [Light obscuration particle count method]

0.2 mL of the sample was injected into a HIAC system (Pacific Scientific Company) to perform measurement of the number of insoluble subvisible particles having a particle size of 1.2 µm or more in 1 mL of the sample.

[0163] Table 6 shows the evaluation results obtained by the light obscuration particle count method in this experiment. It was shown that the number of insoluble subvisible particles increased due to shaking and freezing and thawing, but addition of Polysorbate 80 at a concentration of 0.02 w/v% or more suppressed the increase. It was confirmed that addition of Polysorbate 80 at 0.05 w/v% to the liquid formulation comprising PB009-2 was desirable from the viewpoint of suppressing generation of the insoluble subvisible particles.

[0164] [Table 6]

Table 6

Sample No.	Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)		
	At start of test	After shaking	After freezing and thawing
C-1	1950	N/A (unmeasurable)	61335
C-2	440	2910	3713
C-3	283	2433	8115
C-4	1743	470	11648
C-5	683	4293	15065
C-6	65	228	5938
C-7	275	793	5275

[0165] Experiment 1-6: Selection of optimum pH for stabilizing DFO-anti-human CEACAM5 antibody Fag fragment conjugate

For formulations with the inclusion of PB009-2 at 10 mg/mL and with the inclusion of citric acid at 20 mmol/L or HEPES (2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid) at 20 mmol/L as a buffering agent, sucrose at 10 w/v% as a stabilizer and Polysorbate 80 at 0.05 w/v% as a nonionic surfactant (samples Nos. D-1 to D-8), samples were prepared at a pH of 6.1 to 7.9. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μm , and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap. Thereafter, for each sample, the stability of PB009-2 after storage at 25°C for 1 week in a normally placed state was evaluated on the basis of the amount of multimers measured by the SE-HPLC method. The SE-HPLC method was carried out in the same manner as in Experiment 1-4.

[0166] Table 7 shows the evaluation results obtained by the SE-HPLC method in this experiment. The amount of multimers in SE-HPLC (%) after storage at 25°C for 1 week tended to increase in high-pH samples and low-pH samples, and became the smallest at a pH of about 6.7. Therefore, it was found that the optimum pH was a pH of 6.7, and citric acid

at 20 mmol/L was particularly preferable as a buffering agent for properly maintaining the optimum pH.

[0167] [Table 7]

Table 7

Sample No.	Buffering agent	pH	Amount of multimers in SE-HPLC (%)	
			At start of test	After storage at 25°C for 1 week
D-1	20mmol/L Citric acid	6.1	1.7	3.0
D-2		6.4	1.6	2.9
D-3		6.7	1.5	2.9
D-4		7.0	1.6	3.2
D-5	20mmol/L HEPES	7.0	1.6	3.1
D-6		7.3	1.6	3.3
D-7		7.6	1.7	4.0
D-8		7.9	1.9	4.6

[0168] Experiment 1-7: Examination of optimization of stabilizer concentration for stabilizing DFO-anti-human CEACAM5 antibody Fab fragment conjugate

From liquid formulation formulated to comprise PB009-2 at 10 mg/mL, citric acid at 20 mmol/L and Polysorbate 80 at 0.05 w/v% and have a pH of 6.7 (sample Nos. E-1 to E-5), samples comprising sucrose or glycerin at 0 to 20 w/v% were prepared. After being prepared in accordance with the formulations and compositions, the samples were each aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap. If necessary, hydrochloric acid and/or sodium hydroxide were added as a pH adjuster during preparation of the buffering agent so that a predetermined pH was obtained. Thereafter, for each sample, the stability of PB009-2 after storage at 25°C for 1 week in a normally placed state was evaluated on the basis of the amount of multimers measured by the SE-HPLC method. The SE-HPLC method was carried out in the same manner as in Experiment 1-4.

[0169] Table 8 shows the evaluation results obtained by the SE-HPLC method in this experiment. The results revealed that after storage at 25°C for 1 week, an increase in the amount of multimers in SE-HPLC (%) was suppressed with an increase in the concentration of sucrose or glycerin added. There was no difference in effect on the stability between sucrose and glycerin. The above results showed that sucrose at 10 w/v% was particularly preferable as a pharmaceutical additive for the liquid formulation comprising PB009-2 from the viewpoint of an osmotic pressure ratio.

[0170] [Table 8]

Table 8

Sample No.	Stabilizer	Amount of multimers in SE-HPLC (%)	
		At start of test	After storage at 25°C for 1 week
E-1	None	1.0	3.7
E-2	10w/v% Sucrose	1.0	2.9
E-3	20w/v% Sucrose	0.9	2.6
E-4	10w/v% Glycerin	1.0	3.0
E-5	20w/v% Glycerin	1.0	2.6

[0171] Experiment 1-8: Examination of labeling with ⁸⁹Zr in stabilized liquid formulation comprising DFO-anti-human CEACAM5 antibody Fab fragment conjugate

For the liquid formulation comprising PB009-2 at 10 mg/mL and having formulations described in Table 9 below, labeling efficiency with ⁸⁹Zr after storage at -80°C was evaluated.

⁸⁹Zr was produced as ⁸⁹Zr-Oxalate dissolved in a 1 M aqueous oxalic acid solution at Advanced Science Research Center Okayama University, Department of Radiation Research, Shikada Laboratory. 40 µL of ⁸⁹Zr-Oxalate was neutralized with 20 µL of a 2 M aqueous sodium carbonate solution, and diluted with 190 µL of ultrapure water. Subsequently, 150 µL of a PB009-2 (10 mg/mL) liquid formulation comprising Polysorbate 80 at 0.05 w/v%, sucrose at 10 w/v% or glycerin at 30 w/v% and citric acid at 20 mmol/L was added, and the mixture was reacted at room temperature for 30 minutes. The obtained

reaction mixture was purified using Amicon Ultra 0.5 mL centrifugal filter (Merck Millipore) to obtain a ^{89}Zr -labeled PB009-2 of interest. This ^{89}Zr -DFO-anti-human CEACAM5 antibody Fab fragment conjugate is designated as PB009-3. The PB009-3 solution before and after the purification was measured by TLC (thin-layer chromatography) and the SE-HPLC method to determine the reaction rate of ^{89}Zr . The analysis conditions are as follows.

[0172] The TLC was performed by applying a small amount of the sample to a TLC aluminum sheet (Merck KGaA, 1-05560-0001) and using a 0.1 M EDTA solution (pH: 7.0) as a developing solution. The reaction rate of ^{89}Zr was calculated from:

$$(\text{radiation amount a around origin}) / (\text{total radiation amount b}) \times 100$$

[0173] [Size exclusion chromatography method (SE-HPLC method)]

In SE-HPLC measurement, G3000SWXL (TOSOH CORPORATION) was connected to a HPLC system, and a mobile phase having a composition of phosphoric acid at 20 mmol/L, sodium chloride at 150 mmol/L and 5% acetonitrile (pH 7.0) was fed at a flow rate of 0.5 mL/min. The column temperature was set to 30°C, and detection was performed at UV 280 nm and by RI.

[0174] The test results showed that both the examined formulations gave a high reaction rate value of about 90% (before purification) (Table 9), and comparison between the peak of PB009-3 observed in the UV detector and the RI detector and the peak of PB009-2 observed in the UV detector showed that the retention times of these peaks were equivalent to each other. Therefore, it was confirmed that the DFO-anti-human CEACAM5 antibody Fab fragment conjugate was labeled with ^{89}Zr . It was shown that both the formulation with sucrose or the formulation with glycerin had little possibility of inhibiting the ^{89}Zr labeling reaction.

[0175] [Table 9]

Table 9: Reaction rate determined from TLC results

Formulation	TLC measurement-reaction rate (%)	
	Before purification	After purification
20mmol/L Citric acid, pH6.7, 10w/v% Sucrose, 0.05w/v% Polysorbate 80	87.3	98.9
20mmol/L Citric acid, pH6.7, 30w/v% Glycerin, 0.05w/v% Polysorbate 80	89.1	96.7

[0176] Experiment 1-9: Examination of stability during storage of formulation comprising DFO-anti-human CEACAM5 antibody Fab fragment conjugate

A sample F-1 shown in Table 10 was prepared by adding citric acid, sucrose and Polysorbate 80 to a liquid formulation comprising PB009-2 in such a manner that the final concentration of PB009-2 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. The sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0177] [Table 10]

Table 10

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant
F-1	20mmol/L Citric acid	6.7	10w/v% Sucrose	0.05w/v% Polysorbate 80

[0178] For evaluating the stability of the liquid formulation, a storage stability test of each sample in a normally placed state was conducted. In the storage stability test, the stability of PB009-2 after storage at -20°C or 5°C for 1 to 6 months was evaluated on the basis of the amount of multimers measured by the SE-HPLC method. The SE-HPLC method was carried out in the same manner as in Experiment 1-4.

[0179] Table 11 shows the evaluation results obtained by the SE-HPLC method in this

experiment. The results revealed that there was no problem in storage stability for at least 6 months in storage at -20°C. It was confirmed that in storage at 5°C, storage stability up to 1 month was equivalent to storage stability at -20°C for the same period.

[0180] [Table 11]

Table 11

Sample No.	Storage temperature	Amount of multimers in SE-HPLC (%)			
		At start of test	After storage for 1 month	After storage for 3 months	After storage for 6 months
F-1	-20°C	1.4	2.1	2.3	3.0
F-1	5°C	1.4	2.2	3.1	5.1

[0181] Experiment 2-1: Preparation of anti-human MUC1 antibody Fab fragment

Two anti-human MUC1 antibody Fab fragments designated as P10-1 Fab and P10-2 Fab were prepared. The amino acid sequences of the heavy chain variable regions and the light chain variable regions of P10-1 Fab and P10-2 Fab were specifically designed as sequences expected to improve affinity and not to attenuate affinity even by the binding of a labeling moiety, by using a molecular model of a humanized antibody constructed in accordance with the literature (Proteins, 2014 Aug; 82 (8): 1624-35) after humanization of a 1B2 antibody, which is a mouse-derived anti-human cancer-specific MUC1 antibody, with reference to the method described in the literature (Front Biosci., 2008 Jan 1; 13: 1619-33).

[0182] GS vector pEE6.4 (Lonza Group AG) having an insert of a heavy chain fragment gene formed by connecting a gene encoding a signal sequence (MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 17)) to the 5' side of the heavy chain fragment gene of P10-1 Fab and P10-2 Fab (SEQ ID NO: 5 and SEQ ID NO: 7, respectively) was prepared. Also, GS vector pEE12.4 (Lonza Group AG) having an insert of a light chain gene formed by connecting a gene encoding a signal sequence (MSVPTQVLGLLLLWLTDARC (SEQ ID NO: 18)) to the 5' side of the common light chain gene (SEQ ID NO: 9) of P10-1 Fab and P10-2 Fab was prepared.

[0183] The expression of each Fab fragment was performed by the method of transient

expression. Expi293F cells (Thermo Fisher Scientific Inc.) cultured into approximately 2500000 cells/mL in Expi293 Expression Medium (Thermo Fisher Scientific Inc.) were transfected with the GS vectors of the heavy chain fragment and the light chain mentioned above using ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific Inc.), and cultured for 8 days. After expression, the culture supernatant was purified using KappaSelect (GE Healthcare Japan Corp.) to obtain each Fab fragment.

[0184] The nucleotide sequence of the heavy chain fragment of P10-1 Fab is shown in SEQ ID NO: 5, and the amino acid sequence encoded thereby is shown in SEQ ID NO: 6. The nucleotide sequence of the heavy chain variable region of P10-1 Fab is shown in SEQ ID NO: 11. The amino acid sequence encoded thereby is shown in SEQ ID NO: 12.

[0185] The nucleotide sequence of the heavy chain fragment of P10-2 Fab is shown in SEQ ID NO: 7. The amino acid sequence encoded thereby is shown in SEQ ID NO: 8. The nucleotide sequence of the heavy chain variable region of P10-2 Fab is shown in SEQ ID NO: 13. The amino acid sequence encoded thereby is shown in SEQ ID NO: 14.

[0186] The light chain is common in P10-1 Fab and P10-2 Fab. The nucleotide sequence thereof is shown in SEQ ID NO: 9. The amino acid sequence encoded thereby is shown in SEQ ID NO: 10. The nucleotide sequence of the light chain variable region of P10-1 Fab and P10-2 Fab is shown in SEQ ID NO: 15. The amino acid sequence encoded thereby is shown in SEQ ID NO: 16.

[0187] As a result of analyzing the amino acid modification of purified P10-2 Fab, it was suggested that heavy chain N-terminal glutamine was modified into pyroglutamic acid in a great majority of purified antibodies.

[0188] Experiment 2-2: Labeling of anti-human MUC1 antibody Fab fragment with chelating agent

p-SCN-Bn-DFO (DFO substituted by a p-isothiocyanophenylaminothiocarbonyl group) (Macrocyclics, Inc.) was used in the binding of chelating agent DFO to anti-human MUC1 antibody Fab fragment P10-2. To a Fab fragment solution adjusted to 12.5 mg/mL with phosphate-buffered saline (pH 7.4), a 100 mmol/L aqueous sodium carbonate solution

was added at 10 mmol/L to adjust the pH to 9.0. p-SCN-Bn-DFO was added thereto at a final concentration of 1 mmol/L and reacted at 37°C for 2 hours. Since p-SCN-Bn-DFO has an isothiocyanate group, it rapidly reacts with Lys of the Fab fragment. This was recovered through Amicon Ultra 10K-0.5 mL centrifugal filter to purify a DFO-anti-human MUC1 antibody Fab fragment conjugate bound to DFO via a linker (-C(=S)-NH-(1,4-phenylene)-NH-C(=S)-) (designated as PB010-3).

[0189] Experiment 2-3: Examination of effect of pH on stabilization of DFO-anti-human MUC1 antibody Fab fragment conjugate (effect on multimer generation and insoluble subvisible particle generation)

For liquid formulation comprising PB010-3, an effect of pH on stabilization of PB010-3 was evaluated. In this test, samples G-1 to G-6 shown in Table 12 were prepared by adding a buffering agent, a stabilizer and a nonionic surfactant to the liquid formulation comprising PB010-3 in such a manner that the final concentration of PB010-3 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0190] [Table 12]

Table 12

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant
G-1	20mmol/L Citric acid	6.1	10w/v% Sucrose	0.05w/v% Polysorbate 80
G-2		6.4		
G-3		6.7		
G-4		7.0		
G-5	20mmol/L Phosphoric acid	7.0		
G-6		7.3		

[0191] For evaluating the stability of the liquid formulation, a heat stability test of each

sample in a normally placed state was conducted. In the heat stability test, the stability of PB010-3 after storage at 40°C for 1 week was evaluated on the basis of the amount of multimers measured by the size exclusion chromatography method (SE-HPLC method) and the number of insoluble subvisible particles measured by the light obscuration particle count method. The analysis conditions are as follows.

[0192] [SE-HPLC method]

In SE-HPLC measurement, BioSep SEC s3000 (Phenomenex Inc.) was connected to a HPLC system, PBS (pH 7.4) was used as a mobile phase, and fed at a flow rate of 0.5 mL/min. The injection amount of the sample was 20 µg in terms of PB010-3 (e.g. 10 µL in the case of 2 mg/mL). The column temperature was set to 30°C, the sample temperature was set to 10°C, and detection was performed at UV 280 nm.

[0193] [Light obscuration particle count method]

0.2 mL of the sample was injected into a HIAC system (Pacific Scientific Company) to perform measurement of the number of insoluble subvisible particles of 1.2 µm or more.

[0194] The area of multimers detected by the SE-HPLC method was measured by an automatic analysis method to determine the amount of multimers (%). The amount of multimers is defined in terms of a percentage (%) by measuring the area of multimer peaks detected by the SE-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. Here, the main peak refers to a peak of an active main body (PB010-3 which is not multimerized).

[0195] Table 13 shows the evaluation results obtained by the SE-HPLC method and the light obscuration particle count method in this experiment. The results showed that the amount of multimers in SE-HPLC (%) after storage at 40°C for 1 week tended to increase with an increase in pH. The number of insoluble subvisible particles of 1.2 µm or more after storage at 40°C for 1 week tended to increase with a decrease in pH. Comprehensive judgement on the above results revealed that the optimum pH of the liquid formulation comprising PB010-3 was about 6.5 to 7.0, from the viewpoint of stability.

[0196] [Table 13]

Table 13

Sample No.	Buffering agent	pH	Amount of multimers in SE-HPLC (%)		Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)	
			At start of test	After storage at 40°C for 1 week	At start of test	After storage at 40°C for 1 week
G-1	20mmol/L Citric acid	6.1	0.5	3.4	718	74568
G-2		6.4	0.5	4.1	4970	5660
G-3		6.7	0.5	5.0	1605	4625
G-4		7.0	0.5	6.1	1498	3185
G-5	20mmol/L Phosphoric acid	7.0	0.5	6.3	1708	2143
G-6		7.3	0.5	9.1	920	2288

[0197] Experiment 2-4: Examination of effects of stabilizer and nonionic surfactant on stabilization of DFO-anti-human MUC1 antibody Fab fragment conjugate (effects on multimer generation and insoluble subvisible particle generation)

For liquid formulation comprising PB010-3, effects of various stabilizers on the stability of PB010-3 were evaluated. In this examination, samples H-1 to H-4 shown in Table 14 were prepared by adding a buffering agent, a stabilizer and a nonionic surfactant to the liquid formulation comprising PB010-3 in such a manner that the final concentration of PB010-3 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μm , and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0198] [Table 14]

Table 14

Sample No.	Buffering agent	pH	Nonionic surfactant	Stabilizer
H-1	20mmol/L Citric acid	6.7	None	None
H-2			0.05w/v% Polysorbate 80	None
H-3			0.05w/v% Polysorbate 80	10w/v% Sucrose
H-4			0.05w/v% Polysorbate 80	30w/v% Glycerin

[0199] For evaluating the stability of the liquid formulation, a storage test and a shaking test were conducted on each sample. The storage test was conducted by statically storing each sample under the conditions of 5°C and -20°C. The shaking test was conducted by shaking the sample at 150 rpm for 24 hours. The stability of PB010-3 before and after each test was evaluated on the basis of the amount of multimers measured by the size exclusion chromatography method (SE-HPLC method) and the number of insoluble subvisible particles measured by the light obscuration particle count method. The analysis conditions are the same as in Experiment 2-3.

[0200] Tables 15 and 16 show the evaluation results obtained by the SE-HPLC method and the light obscuration particle count method in this experiment. The results showed that for a formulation without the addition of Polysorbate 80 and a formulation with the addition of sucrose or glycerin in addition to Polysorbate 80, a suppressive effect on increase in the amount of multimers was exhibited even after storage at 5°C or -20°C for 3 months. On the other hand, for the formulation without the addition of Polysorbate 80, the number of insoluble subvisible particles markedly increased after the shaking test. The above results showed that a formulation with the addition of sucrose or glycerin in addition to Polysorbate 80 was desirable, and further, from the viewpoint of an osmotic pressure ratio, sucrose at 10 w/v% was particularly preferable as a pharmaceutical additive for the liquid formulation comprising PB010-3.

[0201] [Table 15]

Table 15

Sample No.	Amount of multimers in SE-HPLC (%)				
	At start of test	After storage at 5°C for 1 month	After storage at 5°C for 3 months	After storage at -20°C for 1 month	After storage at -20°C for 3 months
H-1	0.5	0.6	1.0	0.6	0.7
H-2	0.5	0.7	1.4	0.9	1.3
H-3	0.5	0.6	1.2	0.6	0.9
H-4	0.5	0.6	1.1	0.6	0.8

[0202] [Table 16]

Table 16

Sample No.	Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)	
	At start of test	After shaking
H-1	1988	99380
H-2	1145	1998
H-3	1605	2335
H-4	330	1045

[0203] Experiment 2-5: Examination of effect of surfactant on stabilization of DFO-anti-human MUC1 antibody Fab fragment conjugate (effect on insoluble subvisible particle generation)

For liquid formulation comprising PB010-3, an effect of the surfactant on the stability of PB010-3 was evaluated. In this test, samples I-1 to I-4 shown in Table 17 were prepared by adding a buffering agent, a stabilizer and a nonionic surfactant to the liquid formulation comprising PB010-3 in such a manner that the final concentration of PB010-3 was 10 mg/mL, and adjusting the pH. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μm , and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0204] [Table 17]

Table 17

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant
I-1	20mmol/L Citric acid	6.7	10w/v% Sucrose	0w/v% Polysorbate 80
I-2				0.02w/v% Polysorbate 80
I-3				0.05w/v% Polysorbate 80
I-4				0.1w/v% Polysorbate 80

[0205] For evaluating the stability of the liquid formulation, a shaking test and a freezing and thawing test were conducted on each sample. The shaking test was conducted by

shaking the sample at 150 rpm for 24 hours. The freezing and thawing test was conducted by carrying out a total of three processes each comprising freezing the sample at -80°C for 4 hours or more, and then thawing the sample at 5°C for 4 hours or more. The stability of PB010-3 before and after each test was evaluated on the basis of the number of insoluble subvisible particles measured by the light obscuration particle count method. The analysis conditions are the same as in Experiment 2-3.

[0206] Table 18 shows the evaluation results obtained by the light obscuration particle count method in this experiment. The results revealed that Polysorbate 80 at a concentration of 0.02 w/v% or more had a suppressive effect on increase in the number of insoluble subvisible particles during shaking and freezing and thawing.

[0207] [Table 18]

Table 18

Sample No.	Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)		
	At start of test	After shaking	After freezing and thawing
I-1	12923	77135	18425
I-2	1340	2615	1633
I-3	1933	2168	985
I-4	703	1383	3310

[0208] Experiment 2-6: Examination of labeling with ^{89}Zr in stabilized liquid formulation comprising DFO-anti-human MUC1 antibody Fab fragment conjugate

For the liquid formulation comprising PB010-3 at 10 mg/mL and having formulations described in Table 9 below, labeling efficiency with ^{89}Zr after storage at -80°C was evaluated.

^{89}Zr was produced as ^{89}Zr -Oxalate dissolved in a 1 M aqueous oxalic acid solution at Advanced Science Research Center Okayama University, Department of Radiation Research, Shikada Laboratory. 40 μL of ^{89}Zr -Oxalate was neutralized with 20 μL of a 2 M aqueous sodium carbonate solution, and diluted with 190 μL of ultrapure water. Subsequently,

150 μ L of a PB010-3 (10 mg/mL) liquid formulation comprising Polysorbate 80 at 0.05 w/v%, sucrose at 10 w/v% or glycerin at 30 w/v% and citric acid at 20 mmol/L was added, and the mixture was reacted at room temperature for 60 minutes. The obtained reaction mixture was purified using Amicon Ultra 0.5 mL centrifugal filter (Merck Millipore) to obtain a ^{89}Zr -labeled PB010-3 of interest. This ^{89}Zr -labeled P10-2 Fab DFO (PB010-3) is designated as PB010-4. The PB010-4 solution before and after the purification was measured by TLC (thin-layer chromatography) and the SE-HPLC method to determine the reaction rate of ^{89}Zr . The analysis conditions for the TLC and SE-HPLC method are the same as in Experiment 1-8.

[0209] The test results showed that both the examined formulations gave a high reaction rate value of about 90% (before purification) (Table 19), and comparison between the peak of PB010-4 observed in the UV detector and the RI detector and the peak of PB010-3 observed in the UV detector showed that the retention times of these peaks were equivalent to each other. Therefore, it was confirmed that PB010-3 was labeled with ^{89}Zr . It was shown that both the examined formulations had little possibility of hampering the ^{89}Zr labeling reaction.

[0210] [Table 19]

Table 19: Reaction rate determined from TLC results

Formulation	TLC measurement-reaction rate (%)	
	Before purification	After purification
20mmol/L Citric acid, pH6.7, 10w/v% Sucrose, 0.05w/v% Polysorbate 80	91.2	96.5
20mmol/L Citric acid, pH6.7, 30w/v% Glycerin, 0.05w/v% Polysorbate 80	90.8	99.0

[0211] Experiment 2-7: Examination of stability during storage of formulation comprising DFO-anti-human MUC1 antibody Fab fragment conjugate

For liquid formulation comprising PB010-3, the stability during refrigeration and

freezing storage was evaluated. In this test, a sample J-1 was prepared on the basis of Table 20 by adding a buffering agent, a stabilizer and a nonionic surfactant to the liquid formulation comprising PB010-3 in such a manner that the final concentration of PB010-3 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μ m, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0212] [Table 20]

Table 20

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant
J-1	20mmol/L Citric acid	6.7	10w/v% Sucrose	0.05w/v% Polysorbate 80

[0213] For evaluating the stability of the liquid formulation, each samples was statically stored under the conditions of 5°C and -20°C. The stability of PB010-3 after storage was evaluated on the basis of the amount of multimers measured by the SE-HPLC method, the amount of free Fab bodies (%) measured by the reversed-phase chromatography method (RP-HPLC method) and the number of insoluble subvisible particles measured by the light obscuration particle count method. The analysis conditions are as follows.

[0214] [SE-HPLC method]

The method was carried out under the same conditions as in Experiment 2-3.

[0215] [RP-HPLC method]

In RP-HPLC measurement, Intrada WP-RP (Imtakt Corporation) was connected to a HPLC system, and measurement was performed. 0.1% TFA and 0.1% TFA/acetonitrile were connected to a mobile phase A line and a mobile phase B line, respectively, and fed at a flow rate of 1.0 mL/min while maintaining a ratio as shown in the table below. The injection amount of the sample was 20 μ g in terms of PB010-3 (e.g. 20 μ L in the case of

1 mg/mL). The RP-HPLC gradient program of Table 21 was applied. The column temperature was set to 60°C, the sample temperature was set to 10°C, and detection was performed at UV 214 nm.

[Table 21]

Table 21

Time (min)	A%	B%
0.0	76	24
39.0	63	37
39.1	0	100
41.5	0	100
41.6	76	24
45.0	76	24

[0216] The area of multimers detected by the RP-HPLC method was measured by an automatic analysis method to determine the amount of free Fab bodies (%). The amount of free Fab bodies is defined in terms of a percentage (%) by measuring the area of free Fab fragment peaks detected by the RP-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. In this context, the main peak refers to a peak of an active main body

[0217] [Light obscuration particle count method]

The method was carried out under the same conditions as in Experiment 2-3.

[0218]

Table 22 shows the evaluation results obtained by the SE-HPLC method, the RP-HPLC method and the light obscuration particle count method in this experiment. The results revealed that there was no problem in storage stability for 3 months or less.

[0219] [Table 22]

Table 22

Sample No.	Evaluation item	At start of test	After storage at 5°C for 1 month	After storage at 5°C for 3 months	After storage at -20°C for 1 month	After storage at -20°C for 3 months
J-1	Amount of multimers in SE-HPLC (%)	0.5	0.6	1.2	0.6	0.9
	Amount of free Fab bodies in RP-HPLC (%)	6.6	6.4	6.8	6.4	6.6
	Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)	1605	1775	3883	6383	3353

[0220] Experiment 3-1: Fluorescent labeling of anti-human MUC1 antibody Fab fragment

A fluorescent dye was introduced to P10-2 Fab prepared in Experiment 2-1. Specifically, each Fab fragment solution adjusted to approximately 1 mg/mL with phosphate-buffered saline (pH 7.4) was adjusted to pH 8.5 by the addition of a 1/10 amount of a 1 M dipotassium hydrogen phosphate solution (pH 9). IRDye800CW NHS Ester (LI-COR, Inc.) was added thereto at a final concentration of 310.8 $\mu\text{g/mL}$, and the resultant was stirred at room temperature under shading for 2 hours. IRDye800CW NHS Ester has a N-hydroxysuccinimide group and therefore reacts immediately with Lys of the Fab fragment. This was recovered through Amicon Ultra 3K-0.5 mL centrifugal filter (Merck Millipore) to purify an IRDye800CW-anti-human MUC1 antibody Fab fragment conjugate (designated as PB010-2).

[0221] Experiment 3-2: Effect of pH on stabilization of IRDye800CW-anti-human MUC1 antibody Fab fragment conjugate

For liquid formulation comprising PB010-2, an effect of pH on stabilization of PB010-2 was evaluated. In this test, the concentration of PB010-2 was 10 mg/mL, and samples K-1 to K-5 were prepared on the basis of Table 23. The pH was adjusted by adding an appropriate amount of hydrochloric acid or sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μm , and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0222] [Table 23]

Table 23

Sample No.	Buffering agent	pH
K-1	20mmol/L Citric acid	6.0
K-2		7.0
K-3	20mmol/L Phosphoric acid	6.0
K-4		7.0
K-5		8.0

[0223] For evaluating the stability of the liquid formulation, a heat stability test of each sample in a normally placed state was conducted. In the heat stability test, the stability of PB010-2 after storage at 40°C for 1 week was evaluated on the basis of the amount of multimers measured by the size exclusion chromatography method (SE-HPLC method) and the number of insoluble subvisible particles measured by the microflow imaging method. The analysis conditions are as follows.

[0224] [Size exclusion chromatography method (SE-HPLC method)]

In SE-HPLC measurement, G2000SWXL (TOSOH CORPORATION) was connected to a HPLC system, and a mobile phase having a composition of phosphoric acid at 20 mmol/L and sodium chloride at 1000 mmol/L (pH 7.0) was fed at a flow rate of 0.5 mL/min. The injection amount of the sample was 50 µg in terms of PB010-2 (e.g. 10 µL in the case of 5 mg/mL). The column temperature was set to 30°C, the sample temperature was set to 5°C, and detection was performed at UV 280 nm.

[0225] The area of multimers detected by the SE-HPLC method was measured by an automatic analysis method to determine the amount of multimers (%). The amount of multimers is defined in terms of a percentage (%) by measuring the area of multimer peaks detected by the SE-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. In this context, the main peak refers to a peak of an active main body.

[0226] [Microflow imaging method]

650 μL of the sample was injected into a microflow imaging system (Protein Simple, Inc.) to perform measurement of the number of insoluble subvisible particles having a particle size of 1.2 μm or more in 1 mL of the sample.

[0227] Table 24 shows the evaluation results obtained by the SE-HPLC method and the microflow imaging method in this experiment. The results showed that the amount of multimers in SE-HPLC after storage at 40°C for 1 week tended to increase with an increase in pH. The number of insoluble subvisible particles of 1.2 μm or more after storage at 40°C for 1 week tended to increase with a decrease in pH. Comprehensive judgement on the above results revealed that the optimum pH was about 6.5 to 7.5.

[0228] [Table 24]

Table 24

Sample No.	Buffering agent	pH	Amount of multimers in SE-HPLC (%)		Number of insoluble fine particles ($\geq 1.2 \mu\text{m/mL}$)	
			At start of test	After storage at 40°C for 1 week	At start of test	After storage at 40°C for 1 week
K-1	20 mM Citric acid	6.0	1.4	2.3	10766	163020
K-2		7.0	1.3	3.5	3599	38886
K-3	20 mM Phosphoric acid	6.0	1.3	1.6	13605	130887
K-4		7.0	1.3	4.0	4223	58559
K-5		8.0	1.5	9.9	4318	25668

[0229] Experiment 3-3: Effect of stabilizer or nonionic surfactant on stabilization of IRDye800CW-anti-human MUC1 antibody Fab fragment conjugate

For liquid formulation comprising PB010-2, effects of various stabilizers on the stability of PB010-2 were evaluated. In this test, samples L-1 to L-6 were prepared on the basis of Table 25 by adding a buffering agent and an additive to the liquid formulation comprising PB010-2 in such a manner that the final concentration of PB010-2 was 10 mg/mL. The pH was adjusted by adding an appropriate amount of hydrochloric acid or

sodium hydroxide. Each sample was aseptically filtered through a filter having a pore size of 0.22 μm , and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap.

[0230] [Table 25]

Table 25

Sample No.	Buffering agent	pH	Additive
L-1	20mmol/L Phosphoric acid	7.0	None
L-2			140mmol/L Arginine
L-3			280mmol/L Sucrose
L-4			140mmol/L Sodium chloride
L-5			0.1w/v% Polysorbate 80
L-6			280mmol/L Glycerin

[0231] For evaluating the stability of the liquid formulation, a shaking test, a freezing and thawing test, a heat stability test and a light exposure test on each sample were conducted. The shaking test was conducted by shaking the sample at 150 rpm for 24 hours. The freezing and thawing test was conducted by carrying out a total of three processes each comprising freezing the sample at -80°C for 4 hours or more, and then thawing the sample at 5°C for 4 hours or more. The heat stability test was conducted by storing the sample at 40°C for 2 weeks. The light exposure test was conducted by storing the sample in a horizontally placed state, and applying light of 1000 lx for 96 hours using a white fluorescent lamp. The stability of PB010-2 before and after each test was evaluated on the basis of the amount of multimers measured by the size exclusion chromatography method (SE-HPLC method), the dye antibody ratio measured by the reversed-phase chromatography method (RP-HPLC method) and the number of insoluble subvisible particles measured by the microflow imaging method. The analysis conditions are as follows.

[0232] [Size exclusion chromatography method (SE-HPLC method)]

In SE-HPLC measurement, AdvanceBio SEC 300A (Agilent Technologies) was

connected to a HPLC system, and a mobile phase having a composition of phosphoric acid at 20 mmol/L and sodium chloride at 1000 mmol/L (pH 7.0) was fed at a flow rate of 0.5 mL/min. The injection amount of the sample was 50 µg in terms of PB010-2 (e.g. 10 µL in the case of 5 mg/mL). The column temperature was set to 30°C, the sample temperature was set to 5°C, and detection was performed at UV 280 nm.

[0233] The area of multimers detected by the SE-HPLC method was measured by an automatic analysis method to determine the amount of multimers (%). The amount of multimers is defined in terms of a percentage (%) by measuring the area of multimer peaks detected by the SE-HPLC method using the automatic analysis method, and being divided by the sum of all peak areas including a main peak area. In this context, the main peak refers to a peak of an active main body.

[0234] [Reversed-phase chromatography method (RP-HPLC method)]

In RP-HPLC measurement, Intrada WP-RP (Imtakt Corporation) was connected to a HPLC system, and measurement was performed. 0.1% trifluoroacetic acid/water and 0.1% trifluoroacetic acid/acetonitrile were connected to a mobile phase A line and a mobile phase B line, respectively, and fed at a flow rate of 1.0 mL/min. The injection amount of the sample was 10 µg in terms of PB010-2 (e.g. 10 µL in the case of 1 mg/mL). The RP-HPLC gradient program of Table 26 was applied. The analysis time was 45 minutes, and detection was performed at an UV wavelength of 280 or 780 nm. The column temperature was set to 75°C, and the sample temperature was set to 5°C.

[0235] [Table 26]

Table 26

Time (min)	Mobile phase B%
0.0	20
39.0	60
39.1	100
41.5	100
41.6	20
45.0	20

[0236] The total area of peaks at an UV wavelength of 780 nm and the total area of peaks at an UV wavelength of 280 nm, which peaks had been detected by the RP-HPLC method, the absorbance coefficient of PB010-1 ($1.42 \text{ mL/mg}\cdot\text{cm}^{-1}$), the molecular weight of PB010-1 (47527.43) and the molar absorbance coefficient in the PBS of IRDye800CW ($240000 \text{ mL/mmol}\cdot\text{cm}^{-1}$) were applied to the following calculation formula to determine the dye antibody ratio.

[Expression 1]

$$\text{Dye Antibody Ratio} = \frac{A_{780\text{nm}} \times 1.42 \times 47527.43}{(A_{280\text{nm}} - (A_{780\text{nm}} \times 0.03)) \times 240000}$$

[0237] [Microflow imaging method]

650 μL of the sample was injected into a microflow imaging system (Protein Simple, Inc.) to perform measurement of the number of insoluble subvisible particles having a particle size of 1.0 μm or more in 1 mL of the sample.

[0238] Tables 27 to 29 show the evaluation results obtained by the SE-HPLC method, the RP-HPLC method and the microflow imaging method in this experiment. In a formulation with the addition of arginine and a formulation with the addition of sucrose, an increase in the amount of multimers after storage at 40°C for 2 weeks tended to be suppressed. In a formulation with the addition of arginine, the dye antibody ratio decreased after storage at 40°C for 2 weeks. Further, in a formulation with the addition of sodium chloride and a formulation without the addition of an additive, the number of insoluble subvisible particles

after freezing and thawing tended to increase as compared with other formulations.

Comprehensive judgement on the above results revealed that sucrose was desirable as a stabilizer and a tonicity agent for the liquid formulation comprising PB010-2.

[0239] [Table 27]

Table 27

Sample No.	Additive	Amount of multimers in SE-HPLC (%)				
		At start of test	After shaking	After freezing and thawing	After storage at 40°C for 2 weeks	After light exposure
L-1	None	1.9	2.2	2.5	9.3	3.8
L-2	140mmol/L Arginine	1.9	2.4	2.0	6.2	3.5
L-3	280mmol/L Sucrose	1.8	2.1	1.9	7.9	3.2
L-4	140mmol/L Sodium chloride	1.9	2.3	2.1	9.6	3.7
L-5	0.1w/v% Polysorbate 80	2.1	2.4	2.4	9.4	3.8
L-6	280mmol/L Glycerin	1.9	2.2	1.9	9.0	3.6

[0240] [Table 28]

Table 28

Sample No.	Additive	Dye Antibody Ratio				
		At start of test	After shaking	After freezing and thawing	After storage at 40°C for 2 weeks	After light exposure
L-1	None	2.4	2.4	2.4	1.9	2.3
L-2	140mmol/L Arginine	2.4	2.2	2.4	0.3	1.8
L-3	280mmol/L Sucrose	2.4	2.4	2.4	1.8	2.2
L-4	140mmol/L Sodium chloride	2.5	2.4	2.4	1.8	2.3
L-5	0.1w/v% Polysorbate 80	2.4	2.4	2.5	1.9	2.3
L-6	280mmol/L Glycerin	2.4	2.4	2.4	1.9	2.2

[0241] [Table 29]

Table 29

Sample No.	Additive	Number of insoluble fine particles ($\geq 1.0 \mu\text{m/mL}$)				
		At start of test	After shaking	After freezing and thawing	After storage at 40°C for 2 weeks	After light exposure
L-1	None	1895	16242	30603	12393	3625
L-2	140mmol/L Arginine	1763	2587	13173	8473	3592
L-3	280mmol/L Sucrose	2868	7700	8052	8396	5246
L-4	140mmol/L Sodium chloride	2188	4518	74934	2785	2498
L-5	0.1w/v% Polysorbate 80	1486	4679	11087	5281	3813
L-6	280mmol/L Glycerin	2354	12898	13639	7941	3630

[0242] Experiment 3-4: Selection of optimum pH for stabilizing IRDye800CW-anti-human MUC1 antibody Fag fragment conjugate

For formulations in which citric acid at 20 mmol/L or phosphoric acid at 20 mmol/L is used as a buffering agent in a liquid formulation comprising PB010-2 (sample Nos. M-1 to M-10), samples were prepared at a pH of 6.6 to 7.4. In the sample, the final concentration of PB010-2 was 10 mg/mL, and if necessary, hydrochloric acid and/or sodium hydroxide were added as a pH adjuster during preparation of the buffering agent so that a predetermined pH was obtained. Each sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap. The stability of each sample after the shaking test, the freezing and thawing test, the heat stability test and the light exposure test was evaluated on the basis of the amount of multimers measured by the SE-HPLC method and the dye antibody ratio measured by RP-HPLC. The SE-HPLC method and the RP-HPLC method were carried out in the same manner as in Experiment 3-3.

[0243] Tables 30 and 31 show the results.

In terms of the amount of multimers and the dye antibody ratio, the stability was enhanced as the pH decreased (at a high pH, the stability declined due to heat and light stress). On the other hand, it was found that a pH of 6.8 was particularly preferable because the risk of subvisible particle generation due to a decrease in solubility increased as the pH became closer to 6.0.

[0244] [Table 30]

Table 30

Sample No.	Buffering agent	pH	Amount of multimers in SE-HPLC (%)				
			At start of test	After shaking	After freezing and thawing	After storage at 40°C for 1 week	After light exposure
M-1	20mmol/L Citric acid	6.6	1.8	2.0	1.2	2.9	2.2
M-2		6.8	1.7	2.0	1.2	3.1	2.4
M-3		7.0	1.9	1.9	1.2	3.6	2.4
M-4		7.2	1.7	1.9	1.2	4.0	2.6
M-5		7.4	1.7	1.9	1.2	4.5	2.7
M-6	20mmol/L Phosphoric acid	6.6	1.8	2.1	1.3	3.4	2.6
M-7		6.8	1.8	2.0	1.3	3.8	2.7
M-8		7.0	1.8	2.0	1.5	4.1	2.7
M-9		7.2	1.7	1.9	1.5	4.5	2.8
M-10		7.4	1.8	2.0	1.4	5.4	3.1

[0245] [Table 31]

Table 31

Sample No.	Buffering agent	pH	Dye Antibody Ratio				
			At start of test	After shaking	After freezing and thawing	After storage at 40°C for 1 week	After light exposure
M-1	20mmol/L Citric acid	6.6	1.48	1.48	1.48	1.32	1.36
M-2		6.8	1.49	1.48	1.49	1.30	1.36
M-3		7.0	1.49	1.48	1.49	1.26	1.35
M-4		7.2	1.49	1.47	1.48	1.23	1.34
M-5		7.4	1.49	1.47	1.48	1.19	1.33
M-6	20mmol/L Phosphoric acid	6.6	1.50	1.50	1.49	1.34	1.39
M-7		6.8	1.50	1.49	1.50	1.31	1.38
M-8		7.0	1.51	1.50	1.49	1.27	1.37
M-9		7.2	1.51	1.49	1.49	1.24	1.35

[0246] Experiment 3-5: Effects of buffering agent and surfactant on stabilization of IRDYE800CW-anti-human MUC1 antibody Fab fragment conjugate

For formulations with the inclusion of PB010-2 and with the addition of citric acid at 20 mmol/L (pH 6.8) or phosphoric acid at 20 mmol/L (pH 6.8) and sucrose at 280 mmol/L,

samples with the addition of Polysorbate 80 at 0.05 w/v% as a surfactant and samples without the addition of Polysorbate 80 (sample Nos. N-1 to N-4) were prepared. The final concentration of PB010-2 was 10 mg/mL. After being prepared in accordance with the formulations and compositions, the samples were each aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL). The glass vial was stoppered with a rubber stopper, and covered and capped with an aluminum cap. If necessary, hydrochloric acid and/or sodium hydroxide were added as a pH adjuster during preparation of the buffering agent so that a predetermined pH was obtained.

[0247] The sample was stored at -20°C or 5°C for 1 month, and the heat stability test was conducted by storing the sample in a normally placed state at 40°C for 2 weeks or at 25°C for 1 month. The light exposure test was conducted by storing the sample in a horizontally placed state, and applying light of 1000 lx for 96 hours using a white fluorescent lamp. The stability of PBO10-2 before and after each test was evaluated on the basis of the amount of multimers measured by the SE-HPLC method, the number of insoluble subvisible particles measured by the microflow imaging method, the fluorescence intensity measured by the SE-HPLC method and the antigen binding activity measured by the enzyme-linked immunosorbent assay (ELISA) method. The SE-HPLC method and the microflow imaging method were carried out in accordance with the methods in Experiment 3-3. The fluorescence intensity was evaluated by applying the fluorescence intensity at the detected main peak to the following formula.

[Expression 2]

$$\text{Fluorescence intensity} = \frac{\text{Main peak fluorescence intensity (Ex773/Em792)}}{(A_{280\text{nm}} - (A_{780\text{nm}} \times 0.03))}$$

[0248] [ELISA method]

A phosphate buffer solution containing hMUC-1 (PEPTIDE INSTITUTE, INC.) antigen at 0.8 nM was added to an assay plate, and treated at 2 to 8°C for 18 hours, and the

antigen was then immobilized using tris-buffered saline (TBS) containing 20% Blocking One (nacalai tesque) and Tween-20 at 0.05 w/v%. The PB010-2 solution was stepwise diluted with TBS containing 5% Blocking One and Tween-20 at 0.05 w/v% over a concentration range of 0 to 100000 ng/mL, and added onto the plate having the immobilized antigen. The plate was incubated at 25°C for 60 minutes, and goat anti-human Kappa-HRP (Southern Biotech, Inc.) diluted to 4000 times was then added to the plate. The plate was incubated at 25°C for 60 minutes, and then washed three times. 100 µL of TMB + Substrate-Chromogen (Dako) was added to the plate, incubation was then performed at 25°C for 20 minutes, and sulfuric acid at 1 mol/L was added to stop the reaction. Thereafter, using Spectra Max 190 (Molecular Devices, LLC), an UV absorption at 450 nm was examined to evaluate the binding activity. The binding activity was calculated as a binding activity relative to the activity of PB010-2, which is defined as 100%.

[0249] Tables 32 to 35 show the results.

It was found that use of Polysorbate 80 at 0.05 w/v% as a nonionic surfactant was particularly preferable because in formulations with the addition of Polysorbate 80, an increase in the amount of insoluble subvisible particles after the heat stability test and the light exposure test was suppressed. It was found that use of citric acid as a buffering agent was particularly preferable because in formulations with the addition of citric acid, the increasing tendency in the amount of multimers after storage at 40°C was smaller as compared with formulations with the addition of phosphoric acid. In any of the formulations and storage conditions, there was no decrease in antigen binding activity or the fluorescence intensity.

[0250] [Table 32]

Table 32

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Amount of multimers in SE-HPLC (%)			
					At start of test	After storage at 40°C for 2 weeks	After storage at 25°C for 1 month	After light exposure
N-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0 w/v% Polysorbate 80	2.1	5.6	3.6	3.4
N-2	20mmol/L Citric acid			0.05 w/v% Polysorbate 80	2.1	5.7	3.8	3.5
N-3	20mmol/L Phosphoric acid			0 w/v% Polysorbate 80	2.1	6.6	4.1	3.8
N-4	20mmol/L Phosphoric acid			0.05 w/v% Polysorbate 80	2.2	6.7	4.3	3.9

[0251] [Table 33]

Table 33

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Number of insoluble fine particles ($\geq 1.0 \mu\text{m/mL}$)			
					At start of test	After storage at 40°C for 2 weeks	After storage at 25°C for 1 month	After light exposure
N-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0 w/v% Polysorbate 80	1318	30588	38922	54915
N-2	20mmol/L Citric acid			0.05 w/v% Polysorbate 80	3737	11258	7085	4850
N-3	20mmol/L Phosphoric acid			0 w/v% Polysorbate 80	7532	31998	37638	32926
N-4	20mmol/L Phosphoric acid			0.05 w/v% Polysorbate 80	5228	7911	14801	7486

[0252] [Table 34]

Table 34

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Fluorescence intensity			
					At start of test	After storage at 40°C for 2 weeks	After storage at 25°C for 1 month	After light exposure
N-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0 w/v% Polysorbate 80	0.0526	0.0540	0.0558	0.0521
N-2				0.05 w/v% Polysorbate 80	0.0539	0.0530	0.0546	0.0551
N-3	20mmol/L Phosphoric acid			0 w/v% Polysorbate 80	0.0536	0.0540	0.0551	0.0537
N-4				0.05 w/v% Polysorbate 80	0.0528	0.0532	0.0546	0.0548

[0253] [Table 35]

Table 35

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Antibody binding activity (%)				
					At start of test	After light exposure	After storage at -20°C for 1 month	After storage at 5°C for 1 month	After storage at 25°C for 1 month
N-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0 w/v% Polysorbate 80	117.29	99.94	93.37	100.00	113.16
N-2				0.05 w/v% Polysorbate 80	99.00	91.59	93.61	104.03	104.86
N-3	20mmol/L Phosphoric acid			0 w/v% Polysorbate 80	99.24	96.52	109.16	99.43	122.22
N-4				0.05 w/v% Polysorbate 80	99.59	85.06	108.81	92.74	105.92

[0254] Experiment 3-6. Examination of stability during storage of formulation comprising IRDye800CW-anti-human MUC1 antibody Fab fragment conjugate

A sample with a formulation shown in Table 36 was prepared by adding PB010-2 at a concentration of 10 mg/mL, sucrose at a concentration of 280 mmol/L and Polysorbate 80 at a concentration of 0.05 w/v% to a formulated solution adjusted to a pH of 6.8 using citric acid at 20 mmol/L, and the sample was aseptically filtered through a filter having a pore size of 0.22 µm, and filled in an amount of 1.2 mL into a glass vial (with a volume of 3 mL), and lyophilized. The glass vial was stoppered with a rubber stopper, and covered

and capped with an aluminum cap. Thereafter, the stability of PB010-2 after the heat stability test or the light exposure test was evaluated.

[0255] [Table 36]

Table 36

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant
O-1 (lyophilized product)	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0.05w/v% Polysorbate 80

[0256] The heat stability test was conducted by storing the sample in a normally placed state at 40°C for 2 weeks. The light exposure test was conducted by storing the sample in a horizontally placed state, and applying light of 1000 lx for 96 hours using a white fluorescent lamp. The stability of PB010-2 before and after each test was evaluated on the basis of the amount of multimers and the fluorescence intensity measured by the SE-HPLC method and the dye antibody ratio measured by the RP-HPLC method. The SE-HPLC method and the RP-HPLC method were carried out in accordance with Experiment 3-3. The evaluation of the fluorescence intensity was performed in the same manner as in Experiment 3-5.

[0257] Tables 37 to 39 show the results. The above-described formulations were shown to ensure that PB010-2 is kept stable either after storage at 40°C for 2 weeks or after light exposure.

[0258] [Table 37]

Table 37

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Amount of multimers in SE-HPLC (%)		
					At start of test	After storage at 40°C for 2 weeks	After light exposure
O-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0.05 w/v% Polysorbate 80	2.0	2.1	2.2

[0259] [Table 38]

Table 38

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Dye Antibody Ratio		
					At start of test	After storage at 40°C for 2 weeks	After light exposure
O-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0.05 w/v% Polysorbate 80	1.46	1.46	1.45

[0260] [Table 39]

Table 39

Sample No.	Buffering agent	pH	Stabilizer	Nonionic surfactant	Fluorescence intensity		
					At start of test	After storage at 40°C for 2 weeks	After light exposure
O-1	20mmol/L Citric acid	6.8	280mmol/L Sucrose	0.05 w/v% Polysorbate 80	0.0548	0.0573	0.0558

SEQUENCE LISTING FREE TEXT

[0261] SEQ ID NO: 1: Nucleotide sequence of DNA encoding a PB009-1 Fab heavy chain fragment

SEQ ID NO: 2: Amino acid sequence of PB009-1 Fab heavy chain fragment

SEQ ID NO: 3: Nucleotide sequence of DNA encoding a PB009-1 Fab light chain

SEQ ID NO: 4: Amino acid sequence of PB009-1 Fab light chain

SEQ ID NO: 5: Nucleotide sequence of DNA encoding a P10-1 Fab heavy chain fragment

SEQ ID NO: 6: Amino acid sequence of the P10-1 Fab heavy chain fragment

SEQ ID NO: 7: Nucleotide sequence of DNA encoding a P10-2 Fab heavy chain fragment

SEQ ID NO: 8: Amino acid sequence of the P10-2 Fab heavy chain fragment

SEQ ID NO: 9: Nucleotide sequence of DNA encoding P10-1 Fab and P10-2 Fab light chain

SEQ ID NO: 10: Amino acid sequence of P10-1 Fab and P10-2 Fab light chain

SEQ ID NO: 11: Nucleotide sequence of DNA encoding P10-1 Fab heavy chain variable region

SEQ ID NO: 12: Amino acid sequence of P10-1 Fab heavy chain variable region

SEQ ID NO: 13: Nucleotide sequence of DNA encoding P10-2 Fab heavy chain variable region

SEQ ID NO: 14: Amino acid sequence of P10-2 Fab heavy chain variable region

SEQ ID NO: 15: Nucleotide sequence of DNA encoding P10-1 Fab and P10-2 Fab light chain variable region

SEQ ID NO: 16: Amino acid sequence of P10-1 Fab and P10-2 Fab light chain variable region

SEQ ID NO: 17: Heavy chain signal sequence for PB009-1 Fab, P10-1 Fab and P10-2 Fab

SEQ ID NO: 18: Light chain signal sequence for PB009-1 Fab, P10-1 Fab and P10-2 Fab

SEQ ID NO: 19: Tandem repeat sequence of the extracellular domain of MUC1

CLAIMS

1. A pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, a buffering agent, a stabilizer and a nonionic surfactant and having a pH of 6.5 to 7.5, wherein

the buffering agent comprises citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane, and

the stabilizer comprises sucrose or glycerin.

2. The pharmaceutical composition according to claim 1, wherein

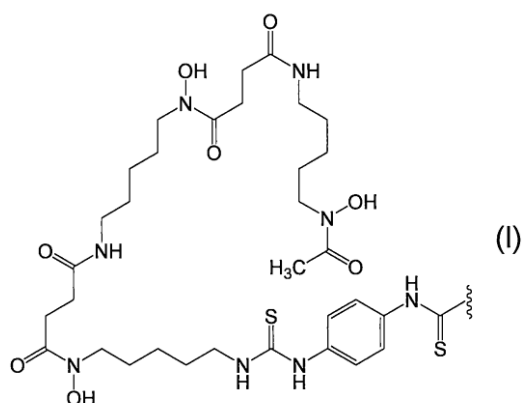
the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of an amino acid sequence from amino acid positions 1 to 121 of SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of an amino acid sequence from amino acid positions 1 to 112 of SEQ ID NO: 4, and

the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 1]



wherein the wavy line represents binding to the anti-human CEACAM5 antibody Fab fragment, where the anti-human CEACAM5 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human CEACAM5 antibody Fab fragment.

3. The pharmaceutical composition according to claim 2, wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4; and

(b) an anti-human CEACAM5 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 2 by the modification of glutamic acid at amino acid position 1 of SEQ ID NO: 2 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 4.

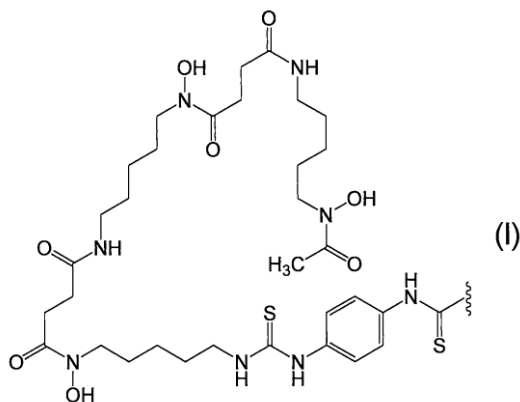
4. The pharmaceutical composition according to claim 1, wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable

region consisting of the amino acid sequence represented by SEQ ID NO: 16; and
(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and

the labeling moiety is a group represented by the following formula (I):

[Chemical Formula 2]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=S) group via an amino group in the anti-human MUC1 antibody Fab fragment.

5. The pharmaceutical composition according to claim 4, wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

- (a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and
- (b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID

NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein the nonionic surfactant comprises Polysorbate 80.

7. The pharmaceutical composition according to any one of claims 2 to 6, wherein the labeling moiety-anti-human antibody Fab fragment conjugate further comprises ⁸⁹Zr.

8. The pharmaceutical composition according to claim 7 for use in the diagnosis of colorectal cancer or a cancer resulting from the metastasis of colorectal cancer.

9. The pharmaceutical composition according to claim 7 for use in the diagnosis of breast cancer or a cancer resulting from the metastasis of breast cancer.

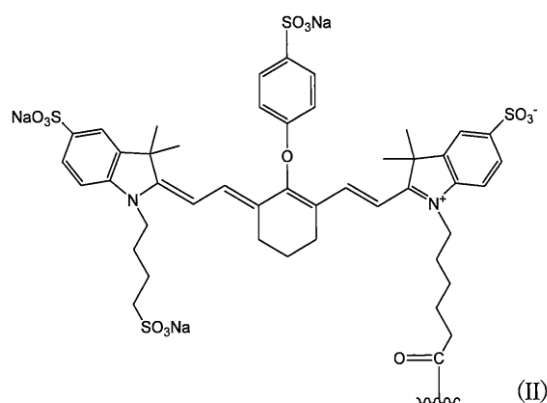
10. The pharmaceutical composition according to claim 1, wherein
the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16; and

(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment comprising a heavy chain variable region derived from a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 12 or SEQ ID NO: 14 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 12 or SEQ ID NO: 14 into pyroglutamic acid, and a light chain comprising a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 16, and

the labeling moiety is a group represented by the following formula (II):

[Chemical Formula 3]



wherein the wavy line represents binding to the anti-human MUC1 antibody Fab fragment, where the anti-human MUC1 antibody Fab fragment is bound to the carbon atom of a labeling moiety terminal C(=O) group via an amino group in the anti-human MUC1 antibody Fab fragment.

11. The pharmaceutical composition according to claim 10, wherein the anti-human antibody Fab fragment is one or more selected from the group consisting of the following (a) and (b):

(a) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10; and

(b) an anti-human MUC1 antibody Fab fragment comprising a heavy chain fragment derived from a heavy chain fragment consisting of the amino acid sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8 by the modification of glutamine at amino acid position 1 of SEQ ID NO: 6 or SEQ ID NO: 8 into pyroglutamic acid, and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 10.

12. The pharmaceutical composition according to claim 10 or 11, wherein the nonionic surfactant comprises Polysorbate 80.

13. The pharmaceutical composition according to any one of claims 10 to 12 for use in the diagnosis of breast cancer or a cancer resulting from the metastasis of breast cancer.

14. The pharmaceutical composition according to claim 13, which is an intraoperative diagnostic drug.

15. The pharmaceutical composition according to any one of claims 1 to 14, wherein the concentration of the buffering agent is 10 to 30 mmol/L.
16. The pharmaceutical composition according to any one of claims 1 to 15, wherein the concentration of the stabilizer is 5 to 30 w/v%.
17. The pharmaceutical composition according to any one of claims 1 to 16, wherein the concentration of the nonionic surfactant is 0.02 to 0.2 w/v%.
18. The pharmaceutical composition according to any one of claims 1 to 17, which is a liquid formulation, a frozen formulation or a lyophilized formulation.
19. A method for producing a pharmaceutical composition comprising a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of:
- (a) producing and adding the labeling moiety-anti-human antibody Fab fragment conjugate;
 - (b) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent;
 - (c) adding sucrose or glycerin as a stabilizer;
 - (d) adding a nonionic surfactant; and
 - (e) adjusting the pH to 6.5 to 7.5.
20. A method for stably preserving a labeling moiety-anti-human antibody Fab fragment conjugate, comprising the steps of:
- (a) adding citric acid, phosphoric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid or trishydroxymethyl aminomethane as a buffering agent to a solution containing the labeling moiety-anti-human antibody Fab fragment conjugate;
 - (b) adding sucrose or glycerin as a stabilizer to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate;
 - (c) adding a nonionic surfactant to the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate; and
 - (d) adjusting the pH of the solution containing the labeling moiety-anti-human antibody Fab fragment conjugate to 6.5 to 7.5.