

- domain of Protein A with any one of amino acid residues selected from the group consisting of an alanine residue (A), a glutamine residue (Q), an asparagine residue (D), a valine residue (V), a serine residue (S), a threonine residue (T), a histidine residue (H), a tyrosine residue (Y), an arginine residue (R), a glutamic acid residue (E), a phenylalanine residue (F), a leucine residue (L), an isoleucine residue (I), and a proline residue (P);
- [4] the method of any one of [1] to [3], wherein the modified immunoglobulin-binding domain is (i) a modified immunoglobulin-binding domain comprising the amino acid sequence of SEQ ID NO: 3 or 5; or (ii) a modified immunoglobulin-binding domain comprising an amino acid sequence in which one to several amino acid residues have been substituted, deleted, added, and/or inserted to the amino acid sequence of SEQ ID NO: 3 or 5 at amino acid residues other than those at positions 4, 7, and 35;
- [5] the method of any one of [1] to [4], wherein the modified immunoglobulin-binding domain has an ability to bind to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R;
- [6] the method of any one of [1] to [5], wherein the Protein A-modified ligand is a modified ligand comprising a modified immunoglobulin-binding domain that consists of at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, and 5;
- [7] the method of any one of [1] to [6], wherein the Protein A-modified ligand is immobilized onto the carrier by any one means selected from the group consisting of (1) to (5) below:
- (1) a method of immobilization onto the carrier through a modified immunoglobulin-binding domain in which 1 to 6 of the amino acid residues at positions 40, 43, 46, 53, 54, and 56 in the C-domain variant or Z-domain of Protein A are additionally substituted with a lysine residue;
 - (2) a method of immobilization onto the carrier through a disulfide bond or a thioether bond by introducing cysteine into the C-terminus of Protein A;
 - (3) a method of immobilization onto an amino group-containing immobilization carrier by cyanation of a thiol group;
 - (4) a method of immobilizing a multimer of modified immunoglobulin-binding domains having a cysteine residue onto an amino group-containing carrier using 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) as a cross-linking agent; and
 - (5) a method of immobilization onto the carrier through a plurality of lysine residues added to the C-terminus of a modified immunoglobulin-binding domain in which the lysine residues at positions 42, 49, 50, and 58 of the C-domain variant of Protein A are substituted with amino acids other than lysine, or to a modified immunoglobulin-binding domain in which the lysine residues at positions 49, 50, and 58 of the Z-domain are substituted with amino acids other than lysine;

Further, the pI values of the antibodies used in the present invention are preferably from 4.0 to 10.0, more preferably from 5.0 to 9.5, and still more preferably from 6.0 to 9.0. The pI values are elevated compared to the pI value of the IgG antibody before the amino acid modification. The isoelectric point of an IgG antibody or the like can be evaluated by a known analysis method such as isoelectric focusing.

[0035]

The amount of binding between a Protein A-modified ligand in the present invention and the Fc region of the IgG antibody to be purified in the present invention is preferably 5 times or more, more preferably 10 times or more, as compared to the amount of binding to unmodified Protein A. The binding amount referred to here is not particularly limited to the method of measurement, and an example thereof includes the method of measuring the dynamic binding capacity described in the Examples herein.

[0036]

Commonly-used Protein A columns specifically include, for example, HiTrap MabSelect SuRe (manufactured by GE Healthcare, trade name), Amsphere A3 (manufactured by JSR Life Sciences, registered trademark), MiniChrom Column Eshmuno A (manufactured by Merck Millipore, registered trademark), MabSpeed rP202 (manufactured by Mitsubishi Chemical, registered trademark), and KanCap Pre-packaged Column (manufactured by Kaneka, trade name).

[0037]

The Protein A affinity column used in the present invention includes an affinity column containing a carrier onto which a Protein A-modified ligand has been immobilized, wherein the Protein A-modified ligand comprises: a modified immunoglobulin-binding domain comprising a modification for substitution of any one or more originally present lysine residues at positions 4, 7, and 35 of the C-domain variant or Z-domain with amino acid residues other than lysine, and also has a binding ability to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R; or a multimer of these modified immunoglobulin-binding domains. This Protein A-modified ligand is characterized in that, when immobilized onto an insoluble carrier *via* its own amino groups, the orientation for maintaining the affinity for immunoglobulin is improved as compared with the unmodified molecule, through the modification of substitution for any one or more lysine residues at positions 4, 7 and 35 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) with other amino acid residues, the C-domain variant being a domain in which the glycine residue at position 29 of the amino acid sequence of the C-domain of Protein A has been substituted with an alanine residue.

[0038]

In addition to the above modifications, the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) may additionally have one to several amino acids substituted to the extent that it has the ability to bind to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R. In particular, in the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2), it is preferred that the number of lysines contained as constituent amino acids is preferably small, and it is desirable that in addition to the above modifications, 1 to 4, preferably 3 or 4, or more preferably 4 of the originally present lysine residues at positions 42, 49, 50 and 58 are substituted with amino acid residues other than lysine.

[0045]

The type of amino acid after substitution at any one or more of positions 42, 49, 50, and 58 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) is not particularly limited, but is preferably alanine, glutamine, asparagine, valine, serine, threonine, histidine, tyrosine, or arginine, and more preferably alanine or arginine.

[0046]

More specifically, if the originally present lysine residue at position 42 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) is substituted with another amino acid residue, the type of amino acid after the substitution is preferably alanine, valine, serine, threonine, or histidine, and more preferably alanine.

[0047]

If the originally present lysine residue at position 49 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) is substituted with another amino acid residue, the type of amino acid after the substitution is preferably arginine, glutamine, asparagine, or tyrosine, and more preferably arginine.

[0048]

If the originally present lysine residue at position 50 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) is substituted with another amino acid residue, the type of amino acid after the substitution is preferably arginine, glutamine, asparagine, or tyrosine, and more preferably arginine.

[0049]

If the originally present lysine residue at position 58 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) is substituted with another amino acid residue, the type of amino acid after the substitution is preferably arginine, glutamine, asparagine, or tyrosine, and more preferably arginine.

[0050]

Furthermore, in the modified immunoglobulin-binding domain in the present invention, in addition to the above modifications, the aspartic acid residue at position 37 of the C-domain

with a lysine residue and immobilizing it onto the carrier through the substituted lysine residue(s).

[0069]

The Protein A affinity columns used in the present invention specifically include AF-
5 rProtein A HC-650F (manufactured by Tosoh), which is an affinity resin in which an Fc-binding
ligand (recombinant of modified Protein A) has been coupled to the synthetic polymer carrier
TOYOPEARL HW-56.

[0070]

The thus prepared carrier onto which a Protein A-modified ligand has been immobilized
10 can be packed in a column to prepare an affinity column (step a). Next, the prepared affinity
column is loaded with a composition containing an IgG antibody comprising modified amino
acid sequence in the CH2 region and CH3 region (step b). In the present invention, the
composition containing an IgG antibody refers to, for example, a culture of IgG antibody-
expressing cells or supernatants thereof. In general, a culture constitutes a complex composition
15 composed of various components such as metabolites of cells as well as various nutrients
necessary for culturing. In order to highly purify the target IgG antibody to the purity required
for pharmaceutical raw materials from that, it is necessary to determine the purification
conditions suitable for the target IgG antibody. The present invention revealed that, to purify an
IgG antibody comprising the amino acid residue substitutions Q311R and P343R in the CH2
20 region and CH3 region, a variant comprising a modification for substitution of any one or more
originally present lysine residues at positions 4, 7, and 35 of the C-domain variant or Z-domain
of Protein A with amino acid residues other than lysine and having an ability to bind to the IgG
antibody is a suitable purification tool.

[0071]

25 The composition containing the IgG antibody can also be pretreated by filtration,
centrifugation or the like before being loaded onto the affinity column. The composition
containing the IgG antibody can be loaded onto the affinity column by a general liquid
chromatography system at an appropriate pressure and flow rate depending on the size and
volume of the column, the size of the carrier, and the like. It is desirable that the amount of the
30 IgG antibody composition loaded onto the affinity column is about the same as the IgG antibody
binding capacity of the affinity column. For example, the binding capacity of the IgG antibody
can be determined by monitoring the concentration of the IgG antibody flowing through from the
affinity column loaded with the composition. More specifically, when the level of the IgG
antibody flowing through from the affinity column becomes the same level as the IgG antibody
35 concentration in the loaded composition, it can be determined that the affinity column condition