Claims

[Claim 1]

A library consisting essentially of a plurality of antibodies each comprising a light chain variable region differing in sequence from each other,
wherein the light chain variable region in each of the antibodies comprises at least one amino acid residue that changes the antigen-binding activity of the antibody depending on calcium ion concentration conditions between a low-calcium concentration selected from the range of 0.1 μM to 30 μM and a high-calcium concentration selected from the range of 100 μM to 10 mM,
wherein the at least one amino acid residue is an amino acid having a calcium-chelating effect selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid, and is located at any one or more of positions 30, 31, and 32 defined by the Kabat numbering in the CDR1 of the light chain variable region, amino acid position 50 defined by the Kabat numbering in the CDR2 of the light chain variable region and the amino acid position 92 defined by the Kabat numbering in the CDR3 of the light chain variable region.

[Claim 2]

The library according to a claim 1, wherein a light chain framework region in the antibody comprises a germline framework sequence.

[Claim 3]

The library according to claim 1 or 2, wherein a heavy chain variable region of the antibody comprises the amino acid sequence of a naive sequence.

[Claim 4]

A library consisting essentially of a plurality of fusion polypeptides each comprising antibodies according to any of claims 1 to 3, wherein each of the fusion polypeptides is a fusion product of a heavy chain variable region of the antibody and at least a portion of a viral coat protein.

[Claim 5]

The library according to claim 4, wherein the viral coat protein is selected from the group consisting of protein pIII, major coat protein pVIII, pVII, pIX, Soc, Hoc, gpD, pv1.

[Claim 6]

A composition comprising a plurality of polynucleotide molecules each encoding antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5.

[Claim 7]

A composition comprising a plurality of vectors each comprising a plurality of polynucleotide molecules according to claim 6 in an operably linked state.

[Claim 8]

The composition according to claim 7, wherein the vectors are replicable expression vectors.

[Claim 9]

The composition according to claim 8, wherein each of the replicable expression vectors is an expression vector in which the polynucleotide is operably linked to a promoter region selected from the group consisting of a lacZ promoter system, an alkaline phosphatase phoA promoter (Ap), a bacteriophage λPL promoter (temperature-sensitive promoter), a tac promoter, a tryptophan promoter, a pBAD promoter, and a bacteriophage T7 promoter.

[Claim 10]

The composition according to claim 8 or 9, wherein each of the replicable expression vectors is an M13, f1, fd, or Pf3 phage or a derivative thereof, or a lambdoid phage or a derivative thereof.

[Claim 11]

A composition comprising a plurality of viruses each comprising vectors according to any of claims 7 to 10.

[Claim 12]

A composition comprising a plurality of viruses each displaying on their surface antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5.

[Claim 13]

A library comprising antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5, wherein the library has 1×10^6 to 1×10^{14} distinct variable region sequences.

[Claim 14]

The library according to claim 13, wherein the library has 1×10^8 or more distinct variable region sequences.

[Claim 15]

A method for preparing a library consisting essentially of a plurality of antibodies each comprising an antibody light chain variable region differing in sequence from each other, the method comprising producing a plurality of antibodies designed so that the light chain variable region in each of the antibodies comprises at least one amino acid residue that changes the antigen-binding activity of the antibody depending on calcium ion concentration conditions between a low-calcium concentration selected from the range of 0.1 μM to 30 μM and a high-calcium concentration selected from the range of 100 μM to 10 mM,

wherein the at least one amino acid residue is an amino acid having a calcium-chelating effect selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid, and is located at any one or more of positions 30, 31, and 32 defined by the Kabat numbering in the CDR1 of the light chain variable region, amino acid position 50 defined by the Kabat numbering in the CDR2 of the light chain variable region and the amino acid position 92 defined by the Kabat numbering in the CDR3 of the light chain variable region.

[Claim 16]

The preparation method according to claim 15, wherein the antibodies are antibodies according to any of claims 1 to 3.

[Claim 17]

The preparation method according to claim 15 or 16, wherein a heavy chain variable region of each of the antibodies is fused with at least a portion of a viral coat protein.

[Claim 18]

The preparation method according to any of claims 15 to 17, wherein the viral coat protein is selected from the group consisting of protein pIII, major coat protein pVIII, pVII, pIX, Soc, Hoc, gpD, pv1.

[Claim 19]

A method for selecting an antibody whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:

a) preparing a library consisting essentially of antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5;

b) contacting the library with antigens under two or more different calcium ion concentration conditions;

c) sorting, from the library, a subpopulation of antibodies whose antigen-binding activity is changed depending on the calcium ion concentration conditions; and

d) isolating each antibody whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the subpopulation sorted in the step c).

[Claim 20]

A method for isolating a polynucleotide encoding an antibody whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:

a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5;
b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antibodies or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;
c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;
d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;
e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d); and
f) isolating the polynucleotides from the isolated virus.

[Claim 21]

The method according to claim 20, wherein the steps c) and d) are additionally repeated at least once.

[Claim 22]

The method according to any one of claims 19 to 21, wherein an antibody having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected.

[Claim 23]

The method according to claim 22, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 24]

The method according to claim 22 or 23, wherein the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 25]

A method for producing an antibody whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:
a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion polypeptides differing in sequence from each other according to claim 4 or 5;
b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antibodies or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;
c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;
d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;
e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d);
f) isolating the polynucleotides from the isolated virus;
g) culturing a host cell transfected with a vector having an operably linked insert of the isolated polynucleotides; and
h) collecting the antibodies from the cultures of the cell cultured in the step g).

[Claim 26]

A method for producing an antibody whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:
a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antibodies differing in sequence from each other according to any of claims 1 to 3 or fusion

polypeptides differing in sequence from each other according to claim 4 or 5;
b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antibodies or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;
c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;
d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;
e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d);
f) isolating the polynucleotides from the isolated virus;
g) linking the isolated polynucleotides in frame with a polynucleotide encoding an antibody constant region;
h) culturing a host cell transfected with a vector having an operably linked insert of the polynucleotides linked in the step g); and
i) collecting the antibodies from the cultures of the cell cultured in the step h).

[Claim 27]

The production method according to claim 25 or 26, wherein the steps c) and d) are additionally repeated at least once.

[Claim 28]

The production method according to any one of claims 25 to 27, wherein an antibody having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected.

[Claim 29]

The production method according to claim 28, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 30]

The production method according to claim 28 or 29, wherein the high-calcium concentration condition is 100 μM to 10 mM.