

[Means for Solving the Problems]

To solve the above-mentioned problems, the present inventors produced substances that neutralize the activity of the bispecific antibody and by targeting the test items that measure the reactivity of FVIII, searched for measurement conditions that ensure accuracy even in the presence of the bispecific antibody. As a result, the present inventors found out that by using neutralizing antibodies against the bispecific antibody at appropriate concentrations (for example, concentrations at which the bispecific antibody can be sufficiently neutralized), the FVIII activity in the plasma of hemophilia A patients can be evaluated accurately by APTT-based one-stage clotting assay, and also found out that the FVIII inhibitor titer in the plasma of a hemophilia A patient carrying the FVIII inhibitor can be evaluated accurately by APTT-based Bethesda assay. Furthermore, the present inventors successfully discovered kits containing neutralizing antibodies against the bispecific antibody having an FVIII-substituting activity for use in the measurement. The present invention is based on these findings and provides the following: In one aspect, the present invention refers to an antibody that neutralizes a bispecific antibody having an activity of functionally substituting for coagulation factor VIII, wherein the bispecific antibody binds to activated coagulation factor IX and coagulation factor X, and the bispecific antibody is the antibody recited below, in which a first polypeptide is associated with a third polypeptide and a second polypeptide is associated with a fourth polypeptide: a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 9, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 11, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 10 (Q499-z121/J327-z119/L404-k), wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to activated coagulation factor IX or an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X, and wherein the neutralizing antibody inhibits the activity of functionally substituting for coagulation factor VIII of the bispecific antibody. In another aspect, the present invention refers to a nucleic acid encoding the antibody of the present invention. In another aspect, the present invention refers to a vector inserted with the nucleic acid of the present invention. In yet another aspect, the present invention refers to a cell comprising the nucleic acid of the present invention or the vector of the present invention. In still another aspect, the present invention refers to a method for producing an antibody by culturing the cell of the present invention. Also described herein is the following:

[1] an antibody that neutralizes a substance having an activity of functionally substituting for coagulation factor VIII;

[2] the antibody of [1], wherein the substance having an activity of functionally substituting for coagulation factor VIII is a bispecific antibody that binds to coagulation factor IX and/or

activated coagulation factor IX and coagulation factor X and/or activated blood coagulation factor X;

[3] the antibody of [2], wherein the bispecific antibody is any one of the antibodies recited below, in which a first polypeptide is associated with a third polypeptide and a second polypeptide is associated with a fourth polypeptide:

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 9, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 11, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 10 (Q499-z121/J327-z119/L404-k);

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 36, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 37, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 38 (Q153-G4k/J142-G4h/L180-k);

[4] the antibody of any one of [1] to [3], wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor IX and/or activated coagulation factor IX, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 12, a CDR2 that consists of the amino acid sequence of SEQ ID NO:

CLAIMS

1. An antibody that neutralizes a bispecific antibody having an activity of functionally substituting for coagulation factor VIII, wherein the bispecific antibody binds to activated coagulation factor IX and coagulation factor X, and the bispecific antibody is the antibody recited below, in which a first polypeptide is associated with a third polypeptide and a second polypeptide is associated with a fourth polypeptide:

a bispecific antibody in which the first polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 9, the second polypeptide is an H chain consisting of the amino acid sequence of SEQ ID NO: 11, and the third polypeptide and the fourth polypeptide are common L chains of SEQ ID NO: 10 (Q499-z121/J327-z119/L404-k), wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to activated coagulation factor IX or an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X, and wherein the neutralizing antibody inhibits the activity of functionally substituting for coagulation factor VIII of the bispecific antibody.

2. The antibody of claim 1, wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor IX and/or activated coagulation factor IX, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 12, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 13, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 14; and

b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 18, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 19, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 20.

3. The antibody of any one of claims 1 to 2, wherein the neutralizing antibody is an antibody comprising

a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 1; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 2.

4. The antibody of claim 1, wherein the neutralizing antibody is an antibody that binds to Fab comprising an antigen-binding site that binds to coagulation factor X and/or activated coagulation factor X, and which comprises

a) a heavy chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 24, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 25, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 26; and

5 b) a light chain variable region comprising a CDR1 that consists of the amino acid sequence of SEQ ID NO: 30, a CDR2 that consists of the amino acid sequence of SEQ ID NO: 31, and a CDR3 that consists of the amino acid sequence of SEQ ID NO: 32.

5. The antibody of any one of claims 1 and 4, wherein the neutralizing antibody is an antibody comprising

10 a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 3; and

b) a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4.

15 6. A nucleic acid encoding the antibody of any one of claims 1 to 5.

7. A vector inserted with the nucleic acid of claim 6.

8. A cell comprising the nucleic acid of claim 6 or the vector of claim 7.

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9. A method for producing an antibody by culturing the cell of claim 8.