

CLAIMS

1. A method for production of a fusion protein in which an anti-transferrin receptor (anti-TfR) antibody and a human iduronate-2-sulfatase (human I2S) are fused, the method comprising;

5 (a) a step of culturing mammalian cells producing the fusion protein in a serum-free medium to let the mammalian cells secrete the fusion protein in the culture medium,

(b) a step of collecting culture supernatant by removing the mammalian cells from the culture medium, and

10 (c) a step of purifying the fusion protein from the culture supernatant, by three column chromatographies consisting of

(i) a column chromatography employing as a solid phase a material to which a substance having affinity for the fusion protein has been bound, wherein the substance having affinity for the fusion protein is selected from the group consisting of Protein A, Protein G, Protein L, Protein A/G, an antigen against said anti-TfR antibody, an antibody recognizing said anti-TfR antibody as an antigen, and an antibody against the human I2S,

15 (ii) a column chromatography employing as a solid phase a material having affinity for the phosphate group, wherein the material having affinity for the phosphate group is fluoroapatite or hydroxyapatite, and

20 (iii) a size exclusion column chromatography, wherein the three column chromatographies (i) to (iii) are performed in this order.

25 2. The method for production according to claim 1, wherein the

substance having affinity for the fusion protein is Protein A

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3. The method for production according to claim 1 or 2, wherein the material having affinity for phosphate group is hydroxyapatite.

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4. The method for production according to any one of claims 1 to 3, wherein said anti-TfR antibody fused to the human I2S is a humanized anti-TfR antibody or a human anti-TfR antibody.

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5. The method for production according to any one of claims 1 to 3, wherein said anti-TfR antibody fused to the human I2S is a humanized anti-TfR antibody.

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6. The method for production according to any one of claims 1 to 5, wherein said anti-TfR antibody is an anti-human transferrin receptor (anti-hTfR) antibody.

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7. The method for production according to any one of claims 1 to 6, wherein said anti-TfR antibody and the human I2S are fused via a linker in the fusion protein, and wherein the linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, copolymer of ethylene glycol and propylene glycol, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ether, biodegradable polymer, lipid polymer, chitin, hyaluronic acid, and biotin-streptavidin,.

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8. The method for production according to any one of claims 1 to 6, wherein the human I2S is linked, by peptide bonds directly or via a linker sequence, to the heavy chain of said anti-TfR antibody on the C-terminal side or the N-terminal side thereof in the fusion protein.

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9. The method for production according to any one of claims 1 to 6, wherein the human I2S is linked, by peptide bonds directly or via a linker sequence, to the light chain of said anti-TfR antibody on the C-terminal side or the N-terminal side thereof in the fusion protein.

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10. The method for production according to claim 8 or 9, wherein the linker sequence consists of 1 to 50 amino acid residues.

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11. The method for production according to claim 10, wherein the linker sequence comprises an amino acid sequence selected from the group consisting of a single glycine, a single serine, the amino acid sequence of Gly-Ser, the amino acid sequence of Gly-Gly-Ser, the amino acid sequence set forth as SEQ ID NO:1, the amino acid sequence set forth as SEQ ID NO:2, the amino acid sequence set forth as SEQ ID NO:3, and the amino acid sequences consisting of 1 to 10 thereof that are consecutively linked.

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12. The method for production according to claim 10 or 11, wherein the linker sequence is represented by the amino acid sequence of Gly-Ser.

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13. The method for production according to any one of claims 1 to 12, wherein the human I2S comprises the amino acid sequence set forth as SEQ ID NO:5.

14. The method for production according to any one of claims 1 to 12, wherein the human I2S has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID:NO5, and has an activity as human I2S.

15. The method for production according to any one of claims 1 to 14, wherein said anti-TfR antibody is a humanized anti-hTfR antibody, and the humanized anti-hTfR antibody is selected from the group consisting of (a) to (c) below;

(a) the humanized anti-hTfR antibody, wherein the light chain and the heavy chain thereof comprise the amino acid sequences set forth as SEQ ID NO:6 and SEQ ID NO:7, respectively,

(b) the humanized anti-hTfR antibody, wherein the light chain and the heavy chain thereof comprise the amino acid sequences set forth as SEQ ID NO:8 and SEQ ID NO:9, respectively, and

(c) the humanized anti-hTfR antibody, wherein the light chain and the heavy chain thereof comprise the amino acid sequences set forth as SEQ ID NO:10 and SEQ ID NO:11, respectively.

16. The method for production according to any one of claims 1 to 14, wherein said anti-TfR antibody is a humanized anti-hTfR antibody,

and the humanized anti-hTfR antibody is selected from the group consisting of (a) to (c) below;

(a) the humanized anti-hTfR antibody, wherein the light chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:6, and wherein the heavy chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:7,

(b) the humanized anti-hTfR antibody, wherein the light chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:8, and wherein the heavy chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:9, and

(c) the humanized anti-hTfR antibody, wherein the light chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:10, and wherein the heavy chain thereof has the amino acid sequence introduced 1 to 3 amino acid substitutions, deletions or additions relative to the amino acid sequence set forth as SEQ ID NO:11.

17. The method for production according to claim 1, wherein said anti-TfR antibody is a humanized anti-hTfR antibody, and the fusion

protein is selected from the group consisting of (a) to (c) below;

(a) the fusion protein comprising the light chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:6, and the heavy chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:7 to which the human iduronate-2-sulfatase set forth as SEQ ID NO:5 is linked on the C-terminal side thereof and via a linker sequence of Gly-Ser,

(b) the fusion protein comprising the light chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:8, and the heavy chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:9 to which the human iduronate-2-sulfatase set forth as SEQ ID NO:5 is linked on the C-terminal side thereof and via a linker sequence of Gly-Ser, and

(c) the fusion protein comprising the light chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:10, and the heavy chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:11 to which the human iduronate-2-sulfatase set forth as SEQ ID NO:5 is linked on the C-terminal side thereof and via a linker sequence of Gly-Ser.

18. The method for production according to claim 1, wherein said anti-TfR antibody is a humanized anti-hTfR antibody, and the fusion protein is selected from the group consisting of (a) to (c) below:

(a) the fusion protein comprising;

the light chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:6, and

the heavy chain of the humanized anti-hTfR antibody to which the human iduronate-2-sulfatase is linked on the C-terminal side thereof via a linker sequence of Gly-Ser, and having the amino acid sequence set forth as SEQ ID NO:12 as a whole,

(b) the fusion protein comprising;

the light chain of the humanized anti-hTfR antibody having the amino acid sequence set forth as SEQ ID NO:8, and

the heavy chain of the humanized anti-hTfR antibody to which the human iduronate-2-sulfatase is linked on the C-terminal side thereof via a linker sequence of Gly-Ser, and having the amino acid sequence set forth as SEQ ID NO:13 as a whole,

(c) the fusion protein comprising;

the light chain of the humanized anti-hTfR antibody has the amino acid sequence set forth as SEQ ID NO:10, and

the heavy chain of the humanized anti-hTfR antibody to which the human iduronate-2-sulfatase is linked on the C-terminal side thereof via a linker sequence of Gly-Ser, and having the amino acid sequence set forth as SEQ ID NO:14 as a whole.