

CLAIMS

Claim 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 Protein A,

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 hydroxyapatite, and

(iii) a size exclusion column chromatography, wherein the three
20 column chromatographies (i) to (iii) are performed in this order, and wherein the light chain and the heavy chain of the anti-TfR antibody comprise the amino acid sequences set forth as SEQ ID NO:10 and SEQ ID NO:11, respectively.

25 Claim 2. The method for production according to claim 1, wherein the anti-TfR antibody fused to the human I2S is a humanized

anti-TfR antibody or a human anti-TfR antibody.

Claim 3. The method for production according to claim 1,
wherein the anti-TfR antibody fused to the human I2S is a humanized
anti-TfR antibody.

Claim 4. The method for production according to any one of
claims 1 to 3, wherein the anti-TfR antibody is an anti-human
transferrin receptor (anti-hTfR) antibody.

Claim 5. The method for production according to any one of
claims 1 to 4, wherein the 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,.

Claim 6. The method for production according to any one of
claims 1 to 4, wherein the human I2S is linked, by peptide bonds
directly or via a linker sequence, to the heavy chain of the anti-TfR
antibody on the C-terminal side or the N-terminal side thereof in the
fusion protein.

Claim 7. The method for production according to any one of

claims 1 to 4, wherein the human I2S is linked, by peptide bonds directly or via a linker sequence, to the light chain of the anti-TfR antibody on the C-terminal side or the N-terminal side thereof in the fusion protein.

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Claim 8. The method for production according to claim 6 or 7, wherein the linker sequence consists of 1 to 50 amino acid residues.

10 Claim 9. The method for production according to claim 8, 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.

15 Claim 10. The method for production according to claim 8 or 9, wherein the linker sequence is represented by the amino acid sequence of Gly-Ser.

20 Claim 11. The method for production according to any one of claims 1 to 10, wherein the human I2S comprises the amino acid sequence set forth as SEQ ID NO:5.

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Claim 12. The method for production according to any one of

claims 1 to 10, 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.

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Claim 13. The method for production according to any one of claims 1 to 12, wherein the anti-TfR antibody is a humanized anti-hTfR antibody, wherein the light chain of the humanized anti-hTfR antibody has, instead of the amino acid sequence set forth as SEQ ID NO:10, 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 of the humanized anti-hTfR antibody has, instead of the amino acid sequence set forth as SEQ ID NO:11, 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.

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Claim 14. The method for production according to claim 1, wherein the anti-TfR antibody is a humanized anti-hTfR antibody, and the fusion protein comprises 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.

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Claim 15. The method for production according to claim 1, wherein the anti-TfR antibody is a humanized anti-hTfR antibody, and the fusion protein comprises;

5 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 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.