

## CLAIMS

1. A method for production of a fusion protein in which an antibody and a human lysosomal enzyme 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

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 antibody, an antibody recognizing said antibody as an antigen, and an antibody against the lysosomal enzyme,

15 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 a size exclusion column chromatography, in this order.

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.

5 4. The method for production according to any one of claims 1 to 3, wherein said antibody fused to the human lysosomal enzyme is a humanized antibody or a human antibody.

10 5. The method for production according to any one of claims 1 to 3, wherein said antibody fused to the human lysosomal enzyme is a humanized antibody.

15 6. The method for production according to any one of claims 1 to 5, wherein said antibody fused to the human lysosomal enzyme recognizes a molecule present on the surface of vascular endothelial cells as an antigen.

20 7. The method for production according to claim 6, wherein the molecule present on the surface of vascular endothelial cells is selected from the group consisting of transferrin receptor (TfR), insulin receptor, leptin receptor, lipoprotein receptor, IGF receptor, OATP-F, organic anion transporter, MCT-8, monocarboxylic acid transporter, and an Fc receptor.

25 8. The method for production according to claim 6, wherein the vascular endothelial cells are cerebral vascular endothelial cells.

9. The method for production according to claim 8, wherein the molecule present on the surface of the cerebrovascular endothelial cell is selected from the group consisting of transferrin receptor (TfR), insulin receptor, leptin receptor, lipoprotein receptor, IGF receptor, OATP-F, organic anion transporter, MCT-8, and monocarboxylic acid transporter.

10. The method for production according to any one of claims 6 to 9, wherein the vascular endothelial cells are human vascular endothelial cells.

11. The method for production according to any one of claims 1 to 10, wherein said antibody is an anti-human transferrin receptor antibody.

12. The method for production according to any one of claims 1 to 11, wherein said antibody and the human lysosomal enzyme 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, biotin-streptavidin, and a derivative thereof.

13. The method for production according to any one of claims 1 to 11, wherein the human lysosomal enzyme is linked, by peptide bonds

directly or via a linker sequence, to the heavy chain of said antibody on the C-terminal side or the N-terminal side thereof in the fusion protein.

14. The method for production according to any one of claims 1 to 11, wherein the human lysosomal enzyme is linked, by peptide bonds directly or via a linker sequence, to the light chain of said antibody on the C-terminal side or the N-terminal side thereof in the fusion protein.

15. The method for production according to claim 13 or 14, wherein the linker sequence consists of 1 to 50 amino acid residues.

16. The method for production according to claim 15, 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.

17. The method for production according to claim 15 or 16, wherein the linker sequence is represented by the amino acid sequence of Gly-Ser.

18. The method for production according to any one of claims 1 to 17, wherein the human lysosomal enzyme is human

iduronate-2-sulfatase (human I2S).

19. The method for production according to claim 18, wherein the human I2S comprises the amino acid sequence set forth as SEQ ID NO:5.

20. The method for production according to claim 18, 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.

21. The method for production according to any one of claims 1 to 20, wherein said 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.

22. The method for production according to any one of claims 1 to

20, wherein said 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.

23. The method for production according to claim 18, wherein said

antibody is a humanized anti-hTfR antibody, the human lysosomal enzyme is human iduronate-2-sulfatase, 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 light chain 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,

24. The method for production according to claim 18, wherein said antibody is a humanized anti-hTfR antibody, the human lysosomal

enzyme is human iduronate-2-sulfatase (human I2S), 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.