What is claimed is:

[Claim 1]

Highly-purified soluble thrombomodulin which has a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of the soluble thrombomodulin, wherein the soluble thrombomodulin is produced by a transformant cell obtained by transfecting a host cell with a DNA containing a nucleotide sequence encoding the soluble thrombomodulin.

[Claim 2]

The highly-purified soluble thrombomodulin according to claim 1, which is ~~industrially~~ manufactured on an industrial scale.

[Claim 3]

The highly-purified soluble thrombomodulin according to claim 1 or 2, which is used as a material for a medicament.

[Claim 4]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 3, wherein purity of the highly-purified soluble thrombomodulin is 99% or higher based on the total proteins.

[Claim 45]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~3~~4, wherein the soluble thrombomodulin is produced by serum-free culture of the transformant cell.

[Claim ~~5~~6]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~4~~5, wherein the host cell is a Chinese hamster ovary cell.

[Claim ~~6~~7]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~5~~6, wherein the soluble thrombomodulin has the following properties (1) to (5):

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~7~~8]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~6~~7, wherein molecular weight of the soluble thrombomodulin is in the range of 50,000 to 80,000.

[Claim ~~8~~9]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~7~~8, wherein the highly-purified soluble thrombomodulin is produced by a method comprising the following steps:

(a) the step of obtaining a transformant cell by transfecting a host cell with a DNA encoding soluble thrombomodulin;

(b) the step of obtaining a solution containing soluble thrombomodulin by culturing the

transformant cell, and

(c) the step of bringing the solution containing soluble thrombomodulin into contact with nylon and/or polyethersulfone to obtain highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin.

[Claim ~~9~~10]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~8~~9, wherein the soluble thrombomodulin is a peptide containing:

(i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and the peptide is soluble thrombomodulin having the following properties (1) to (5):

(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~10~~11]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~8~~9, wherein the soluble thrombomodulin is a peptide containing:

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

and the peptide is soluble thrombomodulin having the properties (1) to (5) mentioned below:

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~11~~12]

The highly-purified soluble thrombomodulin according to any one of claims 1 to ~~8~~9, wherein the DNA containing a nucleotide sequence encoding soluble thrombomodulin is a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11.

[Claim ~~12~~13]

A pharmaceutical composition comprising the highly-purified soluble thrombomodulin according to any one of claims 1 to ~~11~~ 12 and a pharmaceutically acceptable carrier.

[Claim ~~13~~14]

A method for preparing highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, which comprises the step of bringing a solution containing soluble thrombomodulin, which is produced by a transformant cell obtained by transfecting a host cell with a DNA containing a nucleotide sequence encoding soluble thrombomodulin, into contact with nylon and/or polyethersulfone.

[Claim ~~14~~15]

The method according to claim ~~13~~14, wherein the highly-purified soluble thrombomodulin is ~~industrially~~ manufactured on an industrial scale.

[Claim ~~15~~16]

The method according to claim ~~13~~ 14 or ~~14~~15, wherein the highly-purified soluble thrombomodulin is used as a material for a medicament.

[Claim 17]

The method according to any one of claims 14 to 16, wherein purity of the highly-purified soluble thrombomodulin is 99% or higher based on the total proteins.

[Claim ~~16~~18]

The preparation method according to any one of claims ~~13~~ 14 to ~~15~~17, wherein the soluble thrombomodulin is prepared by serum-free culture of the transformant cell.

[Claim ~~17~~19]

The method for preparing highly-purified soluble thrombomodulin according to any one of claims ~~13~~ 14 or ~~16~~18, wherein the soluble thrombomodulin has the following properties (1) to (5);

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~18~~20]

The preparation method according to any one of claims ~~13~~ 14 to ~~17~~19, wherein the host cell is a Chinese hamster ovary cell.

[Claim ~~19~~21]

The preparation method according to any one of claims ~~13~~ 14 to ~~18~~20, wherein

molecular weight of the soluble thrombomodulin is 50,000 to 80,000.

[Claim ~~20~~22]

The preparation method according to any one of claims ~~13~~ 14 to ~~19~~21, wherein the soluble thrombomodulin is a peptide containing:

(i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and the peptide is soluble thrombomodulin having the following properties (1) to (5):

(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~21~~23]

The preparation method according to any one of claims ~~13~~ 14 to ~~19~~21, wherein the soluble thrombomodulin is a peptide containing:

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues, and

the peptide is soluble thrombomodulin having the following properties (1) to (5):

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim ~~22~~24]

The preparation method according to any one of claims ~~13~~ 14 to ~~19~~21, wherein the DNA containing a nucleotide sequence encoding soluble thrombomodulin is a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11.

[Claim ~~23~~25]

The preparation method according to any one of claims ~~13~~ 14 to ~~22~~24, wherein the nylon and/or polyethersulfone is in the form of a filtration membrane.

[Claim ~~24~~26]

The preparation method according to claim ~~23~~25, wherein the filtration membrane has a membrane area of 0.01 to 0.5 m^2 for 1 mg of the host cell-originated proteins.

[Claim ~~25~~27]

The preparation method according to any one of claims ~~13~~ 14 to ~~24~~26, wherein the nylon and/or polyethersulfone is nylon.

[Claim ~~26~~28]

The preparation method according to any one of claims ~~13~~ 14 to ~~25~~27, which is a method for preparing highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, comprising:

(a) the step of obtaining a transformant cell by transfecting a host cell with a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11,

(b) the step of obtaining a solution containing soluble thrombomodulin by culturing the transformant cell,

(c) the step of purifying the solution containing soluble thrombomodulin to a thrombomodulin purity of 99% or higher based on the total proteins, and

(d) the step of bringing the solution containing soluble thrombomodulin into contact with nylon to isolate highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, and

wherein the host cell is a Chinese hamster ovary cell.