

DESCRIPTION

Title of Invention: NOVEL ANTI-HUMAN TIE-2 ANTIBODY

5 Technical Field

[0001]

The present invention relates to a novel anti-human Tie-2 antibody.

Background Art

10 [0002]

A tyrosine kinase with Ig and EGF homology domains 2 (Tie2) is a receptor type tyrosine kinase. Tie2 is mainly known to be expressed in vascular endothelial cells. As the ligand, Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), which are multimer type secreted glycoproteins, are known.

15 [0003]

Ang-1 functions as an agonist for Tie2. It has been found that when Tie2 binds to Ang-1, it is autophosphorylated by forming a multimer and transmits a signal into a cell, thereby promoting an anti-apoptotic action of vascular endothelial cells, vascular stabilization via a permeation inhibitory action of blood vessels, maturation and remodeling (Cell, 1996, Vol. 87, pp. 1171-1180; Genes Dev., 1994, Vol. 8, pp. 1897-1909; Science, 1999, Vol. 286, pp. 2511-2514; and Nat. Struct. Biol., 2003, Vol. 10, pp. 38-44). Further, it has also been known that Ang-1 exerts vasodilating and blood flow-enhancing actions by the production of nitric oxide through Tie2 activation (Pharmacol. Res., 2014, Vol. 80, pp. 43-51). In addition, it is believed that Ang-1 contributes to the stabilization of blood vessels by inhibiting the internalization of vascular endothelial cadherin through Tie2 activation (Dev. Cell, 2008, Vol. 14, pp. 25-36). On the other hand, it is believed that Ang-2 is capable of activating Tie2 on vascular endothelial cells, but its activation is believed to be partial, as compared to Ang-1 (Mol. Cell Biol., 2009, Vol. 29, pp. 2011-2022). Ang-2 binds to the same site of Tie2 with substantially the same affinity as Ang-1, and as a result, it has been suggested that Ang-2 functions as an endogenous Tie2 antagonist from the viewpoint that the activation of Tie2 by Ang-1 is replaced by partial activation of Ang-2 (Science, 1997, Vol. 277, pp. 55-60).

25 [0004]

An increase in the concentration of Ang-2 in the blood has been reported in a disease induced by vascular vulnerability which is considered to be one of the causes of the disease, such as diabetes, diabetic retinopathy, sepsis, and acute renal failure (Atherosclerosis, 2005, Vol. 180, pp. 113-118; Br. J. Ophthalmol., 2004, Vol. 88, pp. 1543-1546; Critical Care, 2009, Vol. 13, p. 207; and Intensive Care Med., 2010, Vol. 36, pp. 462-

470).

[0005]

Regarding relevance to diabetic retinopathy and diabetic macular edema, it has been reported that the concentration of Ang-2 in the blood plasma or the vitreous humor of patients has risen (Br. J. Ophthalmol., 2004, Vol. 88, pp. 1543-1546; and Br. J. Ophthalmol., 2005, Vol. 89, pp. 480-483). Further, in the retinal blood vessel of patients with diabetic retinopathy, the loss of pericytes which are the main Ang-1 producing cells (Cell, 1996, Vol. 87, pp. 1161-1169) has also been known to be one of the characteristic lesions (Retina, 2013, Fifth edition, pp. 925-939). Diabetic macular edema is known for involving the thickening of the macular area as one of the conditions thereof, but it has also been reported that in patients with an increase in the intraocular Ang-1 concentration due to vitreous removal surgery, the thickening of the macular area is decreased (Br. J. Ophthalmol., 2005, Vol. 89, pp. 480-483). Further, from the viewpoints that in retinal edema mouse models with the loss of pericytes in the retinal blood vessels, retinal edema and retinal bleeding are observed, and the pathology onset is inhibited by the intravitreal administration of Ang-1 (J. Clin. Invest., 2002, Vol. 110, pp. 1619-1628), and that in a test using a mouse model with diabetic retinopathy, vascular endothelial cell disorders in the retina are inhibited by the administration of an adenovirus containing a gene encoding Ang-1 (Am. J. Pathol., 2002, Vol. 160, pp. 1683-1693), it has been suggested that Ang-1 has an action of improving the conditions. Meanwhile, it has been reported that in genetically modified mice having Ang-2 specifically over-expressed in the retina, retinal cell damage is increased (Acta. Diabetol. 2010, Vol. 47, pp. 59-64).

[0006]

It has been reported that with regard to critical limb ischemia, the amount of Ang-2 in the blood plasma increases in patients with peripheral arterial diseases, and the amount of Ang-2 expressed in the ischemic limb muscles or the skin tissues in patients with critical limb ischemia is high (J. Am. Coll. Cardiol., 2008, Vol. 52, pp. 387-393; and Int. Angiol., 2011, Vol. 30, pp. 25-34). Moreover, in a test using a rat model with hindlimb ischemia, blood flow recovery and anti-apoptotic effect in the ischemic limb is promoted by the administration of a viral vector containing a gene encoding Ang-1 (Angiogenesis, 2009, Vol. 12, pp. 243-249). From the viewpoint that it has been reported that mature blood vessels covered by the smooth muscle cells are increased in the border zone of infarcted area by the administration of a virus containing a gene encoding Ang-1 in a coronary artery ligation model of a db/db mouse as an animal model with type 2 diabetes (Diabetes, 2008, Vol. 57, pp. 3335-3343), an effect of promoting the maturation of unstable neovascular vessels can be expected by the activation of Tie2 signals.

[0007]

As an antibody showing an agonistic action on a human Tie2, a murine

monoclonal antibody 15B8 (Patent Document 1) has been reported. It has been reported that 15B8 binds to the human Tie2 to induce an anti-apoptotic action in a human vascular endothelial cell HUVEC (Patent Document 1)

5 Related Art
 Patent Document

 [0008]

 [Patent Document 1] WO 2000/018804

10 Disclosure of Invention
 Problems to Be Solved by the Invention

 [0009]

 An object of the present invention is to provide an anti-human Tie2 antibody for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb
15 ischemia by binding to a human Tie2 to activate the human Tie2.

 Means for Solving the Problems

 [0010]

 The present inventors have repeatedly conducted substantial and inventive studies
20 in preparation of an anti-human Tie2 antibody, and as a result, they have found that a tetravalent anti-human Tie2 antibody comprising a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4 is prepared (Examples 1 to 8), and thus, the anti-
25 human Tie2 antibody binds to the human Tie2 (Example 12), induces the anti-apoptotic action in a human Tie2-expressing BaF3 cell (Examples 9 and 11), and inhibits the vascular hyperpermeability in a rat model with vascular hyperpermeability (Examples 10 and 13). As a result, they have provided such an anti-human Tie2 antibody, thereby completing the present invention.

30 [0011]

 That is, the present invention may include the following invention as a material or a method which is medically or industrially applicable.

 [1] An anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, wherein

35 the heavy chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of the amino acid numbers 99 to 111 of SEQ ID NO:

2;

the light chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3
 5 consisting of the amino acid sequence of the amino acid numbers 94 to 102 of SEQ ID NO: 4; and

the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites.

10 [2] The anti-human Tie2 antibody or the antigen-binding fragment thereof of [1], selected from (1) or (2) below:

(1) an anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, in which

15 the heavy chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2,

the light chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and

20 the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites; and

(2) an anti-human Tie2 antibody or an antigen-binding fragment thereof which is an antibody or an antigen-binding fragment thereof derived from posttranslational modification of the anti-human Tie2 antibody or the antigen-binding fragment thereof of
 25 (1).

[3] The anti-human Tie2 antibody of [1], wherein

the antibody comprises two heavy chains and four light chains;

30 each heavy chain comprises two structures consisting of a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of the amino acid numbers 99 to 111 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the carboxy terminus (C terminus) of one of the structures is linked to the amino terminus (N terminus) of the other structure through a linker; and

35 each light chain comprises a light chain variable region comprising CDR1 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of the amino acid

numbers 94 to 102 of SEQ ID NO: 4, and a light chain constant region.

[4] The anti-human Tie2 antibody of [3], selected from (1) or (2) below:

(1) an anti-human Tie2 antibody comprising two heavy chains and four light chains, in which

each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker; and

each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region; and

(2) an anti-human Tie2 antibody, which is an antibody derived from posttranslational modification of the anti-human Tie2 antibody of (1).

[5] The anti-human Tie2 antibody of [4], wherein

the anti-human Tie2 antibody comprises two heavy chains and four light chains; each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker; and

each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region.

[6] An anti-human Tie2 antibody which is an antibody derived from posttranslational modification of the anti-human Tie2 antibody of [5].

[7] The anti-human Tie2 antibody of [6], wherein the posttranslational modification is pyroglutamylation at the N terminus of the heavy chain variable region and/or deletion of lysine at the C terminus of the heavy chain.

[8] The anti-human Tie2 antibody of any one of [3] to [7], comprising a heavy chain constant region which is a human Igγ1 constant region or a human Igγ4 constant region.

[9] The anti-human Tie2 antibody of [8], in which the human Igγ1 constant region is a human Igγ1 constant region having amino acid variations of L234A, L235A, and P331S, or a human Igγ1 constant region having amino acid variations of L234A, L235A, P331S, and I253A.

[10] The anti-human Tie2 antibody of [8], in which the human Igγ4 constant region is a human Igγ4 constant region having amino acid variations of S228P and L235E.

[11] The anti-human Tie2 antibody of any one of [3] to [7], comprising a light chain constant region which is a human Igκ constant region.

[12] The anti-human Tie2 antibody of any one of [3] to [7], comprising a heavy chain

constant region which is a human Igγ1 constant region or a human Igγ4 constant region and a light chain constant region which is a human Igκ constant region.

[13] The anti-human Tie2 antibody of [12], in which the human Igγ1 constant region is a human Igγ1 constant region having amino acid variations of L234A, L235A, and P331S, or
5 a human Igγ1 constant region having amino acid variations of L234A, L235A, P331S, and I253A.

[14] The anti-human Tie2 antibody of [12], in which the human Igγ4 constant region is a human Igγ4 constant region having amino acid variations of S228P and L235E.

[15] The anti-human Tie2 antibody of any one of [3] to [7], in which the linker is a peptide
10 linker comprising 5 to 70 amino acids.

[16] The anti-human Tie2 antibody of [15], in which the linker comprises the amino acid sequence of a hinge region or a portion thereof.

[17] The anti-human Tie2 antibody of [16], in which the linker comprises the amino acid sequence shown by SEQ ID NO: 13.

[18] The anti-human Tie2 antibody of [4], comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.
15

[19] The anti-human Tie2 antibody of [4], comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 6 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.
20

[20] The anti-human Tie2 antibody of [4], comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 10 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[21] An anti-human Tie2 antibody which is an antibody derived from posttranslational
25 modification of the anti-human Tie2 antibody of any one of [18] to [20]

[22] The anti-human Tie2 antibody of [21], wherein the posttranslational modification is pyroglutamylation at the N terminus of the heavy chain variable region and/or deletion of lysine at the C terminus of the heavy chain.

[23] The anti-human Tie2 antibody of [21], comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.
30

[24] A tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof, binding to the same human Tie2 epitope as the anti-human Tie2 antibody of [18] or [23].

[25] The tetravalent anti-human Tie2 antibody or the antigen-binding fragment thereof of [24], wherein the human Tie2 epitope is the human Tie2 epitope containing the amino acid of the amino acid numbers 192, 195 and 197 of Accession No. NP_000450.2.
35

[26] A polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2].

[27] A polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2].

[28] A polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20].

5 [29] A polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody of any one of [18] to [20].

[30] An expression vector comprising the polynucleotide of [26] and/or [27].

[31] An expression vector comprising the polynucleotide of [28] and/or [29].

[32] A host cell transformed with the expression vector of [30], which is selected from the group consisting of (a) to (d) below:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2], and a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or an antigen-binding fragment thereof;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2] and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or an antigen-binding fragment thereof;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2]; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2].

[33] A host cell transformed with the expression vector of [31], selected from the group consisting of (a) to (d) below:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20] and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20] and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of

any one of [18] to [20]; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody of any one of [18] to [20].

- 5 [34] A method for producing an anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising culturing host cell(s) selected from the group consisting of (a) to (c) below to express a tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof:

10 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2] and a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof;

15 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2] and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof; and

20 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of [2] and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof.

- 25 [35] A method for producing an anti-human Tie2 antibody, comprising culturing host cell(s) selected from the group consisting of (a) to (c) below to express an anti-human Tie2 antibody:

30 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20] and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20] and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

35

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of any one of [18] to [20] and a host cell transformed with an expression vector comprising a

polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody.

[36] An anti-human Tie2 antibody or an antigen-binding fragment thereof, produced by the method of [34].

5 [37] An anti-human Tie2 antibody produced by the method of [35].

[38] A pharmaceutical composition comprising the anti-human Tie2 antibody or the antigen-binding fragment thereof of any one of [1] to [23], [36], and [37], and a pharmaceutically acceptable excipient.

[39] A pharmaceutical composition comprising the anti-human Tie2 antibody of [5], the anti-human Tie2 antibody of [6], and a pharmaceutically acceptable excipient.

[40] A pharmaceutical composition comprising the anti-human Tie2 antibody of [18], the anti-human Tie2 antibody of [23], and a pharmaceutically acceptable excipient.

[41] The pharmaceutical composition of any one of [38] to [40], which is a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

[42] A method for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia, comprising administering a therapeutically effective amount of the anti-human Tie2 antibody or the antigen-binding fragment thereof of any one of [1] to [23], [36], and [37].

20 [43] The anti-human Tie2 antibody or the antigen-binding fragment thereof of any one of [1] to [23], [36], and [37], for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

[44] Use of the anti-human Tie2 antibody or the antigen-binding fragment thereof of any one of [1] to [23], [36], and [37] for manufacture of a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

[0012]

The anti-human Tie-2 antibody or the antigen-binding fragment thereof includes a fusion of the antibody with another peptide or protein, and a modification having a modifying agent bound thereto.

Effects of the Invention

[0013]

The anti-human Tie2 antibody of the present invention can be used as an agent for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia by binding to a human Tie2 to activate the human Tie2.

Brief Description of Drawings

[0014]

Fig. 1 shows an example of the format of a tetravalent anti-human Tie2 antibody of the present invention.

[0015]

5 Fig. 2 shows the vascular permeability inhibitory action of the fully human 2-16A2 and TIE-1-Ig γ 1-WT in a rat model with vascular permeability. The vertical axis indicates the amount of leakage of an Evans Blue dye (****: $p < 0.0001$ vs a vehicle group).

[0016]

10 Fig. 3 shows the vascular permeability inhibitory action of TIE-1-Ig γ 1-LALA in a rat model with vascular permeability. The vertical axis indicates the amount of leakage of an Evans Blue dye (****: $p < 0.0001$ vs a vehicle group).

[0017]

15 Fig. 4 shows the retinal edema inhibitory action of TIE-1-Ig γ 1-LALA in a mouse model with the loss of pericytes in the retinal blood vessel. The vertical axis indicates a sum of a retinal nerve fiber layer and a retinal ganglion cell layer (##: $p < 0.005$ vs Cont. group, *: $p < 0.05$ vs Veh. group).

[0018]

20 Fig. 5 shows the blood flow improving action of TIE-1-Ig γ 1-LALA in a mouse model with hindlimb ischemia. The vertical axis indicates the amount of blood flow. (*: $p < 0.05$ vs control group, **: $p < 0.01$ vs control group).

[0019]

25 Fig. 6 shows a representative example of the results of a surface plasmon resonance phenomenon as epitope analysis of TIE-1-Ig γ 1-LALA. The vertical axis indicates a binding responsiveness (Resonance Unit: RU) and the horizontal axis indicates time (seconds).

[0020]

30 Fig. 7 shows the results of ELISA as epitope analysis of TIE-1-Ig γ 1-LALA. The vertical axis indicates a luminescent intensity and the horizontal axis indicates a concentration of TIE-1-Ig γ 1-LALA (ng/mL).

Embodiments for Carrying Out the Invention

[0021]

Hereinafter, the present invention will be described in detail.

35 [0022]

There are five classes of IgG, IgM, IgA, IgD, and IgE in an antibody. The basic structure of an antibody molecule is configured of heavy chains having a molecular weight of 50000 to 70000 and light chains having a molecular weight of 20000 to 30000 in each

of the classes in common. Heavy chain usually consists of a polypeptide chain comprising approximately 440 amino acids, has a distinctive structure for each of the classes, and is referred to as $I\gamma$, $I\mu$, $I\alpha$, $I\delta$, and $I\epsilon$ corresponding to IgG , IgM , IgA , IgD , and IgE , respectively. Further, four subclasses of $IgG1$, $IgG2$, $IgG3$, and $IgG4$ are present in IgG , and the heavy chains respectively corresponding thereto are referred to as $I\gamma1$, $I\gamma2$, $I\gamma3$, and $I\gamma4$. Light chain usually consists of a polypeptide chain comprising approximately 220 amino acids, two types of which, type L and type K are known, and are referred to as $I\lambda$ and $I\kappa$. In a peptide configuration of the basic structure of antibody molecules, two homologous heavy chains and two homologous light chains are bound by disulfide bonds (S-S bond) and non-covalent bonds, and the molecular weight thereof is 150000 to 190000. Two kinds of light chains can be paired with any heavy chain.

[0023]

With regard to intrachain S-S bonds, four of the S-S bonds are present in the heavy chain (five in $I\mu$ and $I\epsilon$) and two of them are present in the light chain; one loop is formed per 100 to 110 amino acid residues, and this steric structure is similar among the loops and are referred to as a structural unit or a domain. The domain located at the amino-terminal side (N terminal side) in both of the heavy chain and the light chain, whose amino acid sequence is not constant even in a case of a sample from the same class (subclass) of the same kind of animal is referred to as a variable region, and respective domains are referred to as a heavy chain variable region and a light chain variable region. The amino acid sequence of the carboxy-terminal side (C terminal side) from the variable region is nearly constant in each class or subclass and is referred to as a constant region.

[0024]

An antigen binding site of an antibody is configured of heavy chain variable region (VH) and the light chain variable region (VL), and the binding specificity depends on the amino acid sequence of this site. On the other hand, biological activities such as binding to complements and various cells reflect differences in the constant region structures among each class Ig . It is understood that the variability of variable regions of the light chains and the heavy chains is mostly limited to three small hypervariable regions present in both chains and these regions are referred to as complementarity determining regions (CDR: CDR1, CDR2, and CDR3 from the N terminal side). The remaining portion of the variable region is referred to as a framework region (FR) and is relatively constant.

[0025]

With regard to the constant region, the heavy chain constant region consists of three regions, which are each called a CH1 region, a CH2 region, and a CH3 region in order from the variable region side. The light chain constant region consists of one region. A peptide sequence called a hinge region is present between the CH1 region and

the CH2 region. The hinge region contributes to the mobility of a structure consisting of the heavy chain variable region and the CH1 region.

[0026]

Further, various kinds of antigen-binding fragments comprising VH and VL of an antibody have antigen binding activity. For example, a single-chain variable region fragment (scFv), Fab, Fab', and F(ab')₂ are exemplified as typical antigen-binding fragments. A Fab is a monovalent antigen-binding fragment which is constituted with a light-chain and a heavy-chain fragment comprising a VH, a CH1 region, and a portion of the hinge region. A Fab' is a monovalent antigen-binding fragment which is constituted with a light-chain and a heavy-chain fragment comprising a VH, a CH1 region, and a portion of the hinge region, and cysteine residues constituting the inter-heavy-chain S-S bond are comprised in the portion of the hinge region. A F(ab')₂ is a bivalent antigen-binding fragment having a dimeric structure in which two Fab' fragments bind to each other via the inter-heavy-chain S-S bond in the hinge region. An scFv is a monovalent antigen-binding fragment which is constituted with a VH and VL connected with a linker peptide.

[0027]

An antibody having two or more antigen-binding sites is referred to as a multivalent antibody. Among these, an antibody having four antigen-binding sites is referred to as a tetravalent antibody. For the tetravalent antibody, various formats (structures) have been reported (Nat. Rev. Immunol. 2010, Vol. 10, pp. 301-316; J. Immunol., 2003, Vol. 170, pp. 4854-4861; Mol. Immunol., 2000, Vol. 37, pp. 1067-1077; Biochem. J., 2007, Vol. 406, pp. 237-246; and J. Immunol. Methods, 2003, Vol. 279, pp. 219-232). For example, a tetravalent antibody in which the N terminals of a heavy chain variable region and a light chain variable region of a bivalent antibody are each linked to the C terminals of the heavy chain variable region and the light chain variable region through a linker; a tetravalent antibody comprising two heavy chains and four light chains, in which each heavy chain comprises two structures consisting of a heavy chain variable region and a CH1 region; a tetravalent antibody in which the C terminals of scFv are bonded to each streptavidin of a tetrameric streptavidin one by one; a tetravalent antibody in which the C terminals of scFv are bonded to each p53 of a tetrameric p53 one by one; and a tetravalent antibody in which the N terminals of a CH3 region are linked to the C terminals of a dimeric scFv through a linker have been reported.

[0028]

<Anti-Human Tie2 Antibody of the Present Invention>

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention includes an anti-human Tie2 antibody or an antigen-binding fragment thereof, having the following characteristics.

An anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, in which

the heavy chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2,

5 the light chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and

the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites.

10 [0029]

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is not particularly limited as long as it is a tetravalent antibody, and various formats of tetravalent antibodies described in, for example, Nat. Rev. Immunol. 2010, Vol. 10, pp. 301-316, J. Immunol., 2003, Vol. 170, pp. 4854-4861; Mol. Immunol., 15 2000, Vol. 37, pp. 1067-1077; Biochem. J., 2007, Vol. 406, pp. 237-246; J. Immunol. Methods, 2003, Vol. 279, pp. 219-232; and the like can be used for the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention.

[0030]

20 Preferably, the anti-human Tie2 antibody of the present invention comprises two heavy chains and four light chains,

each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker, and

25 each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region.

Hereinafter, a tetravalent antibody in the format is referred to as a tandem antibody, and an example thereof is shown in Fig. 1.

30 [0031]

In the case where the anti-human Tie2 antibody of the present invention is a tandem antibody, a constant region (for example, a constant region of Igγ1, Igγ2, Igγ3 or Igγ4 as a heavy chain constant region, and a constant region of Igλ or Igκ as a light chain constant region) in any subclass can be selected as the constant region. The heavy chain constant region (including a CH1 region, a CH2 region, and a CH3 region) is preferably a human Igγ1 constant region or a human Igγ4 constant region. The light chain constant region is preferably a human Igκ constant region.

[0032]

In the case where a human Igγ1 constant region is used as the heavy chain constant region of the anti-human Tie2 antibody of the present invention, examples of the CH1 region, the CH2 region, and the CH3 region of the human Igγ1 constant region comprise a CH1 region consisting of the amino acid sequence of the amino acid numbers 350 to 447 of SEQ ID NO: 8, a CH2 region consisting of the amino acid sequence of the amino acid numbers 463 to 572 of SEQ ID NO: 8, and a CH3 region consisting of the amino acid sequence of the amino acid numbers 573 to 679 of SEQ ID NO: 8.

[0033]

In the case where a human Igγ1 constant region is used as the heavy chain constant region of the anti-human Tie2 antibody of the present invention, a human Igγ1 constant region having introduction of amino acid variation, such as L234A (having substitution of leucine at the amino acid 234th position with alanine according to an EU index such as Kabat), L235A (having substitution of leucine at the amino acid 235th position with alanine according to an EU index such as Kabat), and P331S (having substitution of proline at the amino acid 331st position with serine according to an EU index such as Kabat) can also be used in order to reduce the antibody-dependent cellular cytotoxicity or the complement-dependent cytotoxicity activity of an antibody (Mol. Immunol., 1992, Vol. 29, No.5, pp. 633-639). Further, from the viewpoint of pharmacokinetics, a human Igγ1 constant region to which amino acid variations has been introduced, such as I253A (having substitution of isoleucine at the amino acid 253th position with alanine according to an EU index such as Kabat) can also be used in order to attain a rapid loss in the blood (J. Immunol., 1997, Vol. 158, pp. 2211-2217). The residue numbers with respect to the introduction of amino acid variation in the constant region of the antibody used in the present specification are in accordance with an EU index (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institute of Health, Bethesda).

[0034]

In the case where a human Igγ1 constant region is used as the heavy chain constant region of the anti-human Tie2 antibody of the present invention, the human Igγ1 constant region is preferably a human Igγ1 constant region having amino acid variations of L234A, L235A, and P331S, or L234A, L235A, P331S and I253A. Examples of the CH1 region, the CH2 region, and CH3 region of the human Igγ1 constant region having amino acid variations of L234A, L235A, and P331S comprise a CH1 region consisting of the amino acid sequence of the amino acid numbers 350 to 447 of SEQ ID NO: 2, a CH2 region consisting of the amino acid sequence of the amino acid numbers 463 to 572 of SEQ ID NO: 2, and a CH3 region consisting of the amino acid sequence of the amino acid numbers 573 to 679 of SEQ ID NO: 2. Examples of the CH1 region, the CH2 region, and the CH3 region of the human Igγ1 constant region having amino acid variations of L234A, L235A,

P331S, and I253A comprise a CH1 region consisting of the amino acid sequence of the amino acid numbers 350 to 447 of SEQ ID NO: 6, a CH2 region consisting of the amino acid sequence of the amino acid numbers 463 to 572 of SEQ ID NO: 6, and a CH3 region consisting of the amino acid sequence of the amino acid numbers 573 to 679 of SEQ ID NO: 6.

[0035]

In the case where a human Igγ4 constant region is used as the heavy chain constant region of the anti-human Tie2 antibody of the present invention, a human Igγ4 constant region having introduction of amino acid variations such as S228P (having substitution of serine at the amino acid 228th position with proline according to an EU index such as Kabat) and L235E (having substitution of leucine at the amino acid 235th position with glutamic acid according to an EU index such as Kabat) can also be used in order to inhibit Fab arm exchange (Drug Metab. Dispos., 2010, Vol. 38, No.1, pp. 84-91).

[0036]

In the case where a human Igγ4 constant region is used as the heavy chain constant region of the anti-human Tie2 antibody of the present invention, the human Igγ4 constant region is preferably a human Igγ4 constant region having amino acid variations of S228P and L235E. Examples of the CH1 region, the CH2 region, and the CH3 region of the human Igγ4 constant region having amino acid variations of S228P and L235E comprise a CH1 region consisting of the amino acid sequence of the amino acid numbers 350 to 447 of SEQ ID NO: 10, a CH2 region consisting of the amino acid sequence of the amino acid numbers 460 to 569 of SEQ ID NO: 10, and a CH3 region consisting of the amino acid sequence of the amino acid numbers 570 to 676 of SEQ ID NO: 10.

[0037]

Examples of the human Igk constant region include a human Igk constant region consisting of the amino acid sequence of the amino acid numbers 114 to 219 of SEQ ID NO: 4.

[0038]

Preferably, in the case where the anti-human Tie2 antibody of the present invention is a tandem antibody, the heavy chain constant region is a human Igγ1 constant region or a human Igγ4 constant region, and the light chain constant region is a human Igk constant region. In the case where the heavy chain constant region is a human Igγ1 constant region, the human Igγ1 constant region is preferably a human Igγ1 constant region having amino acid variations of L234A, L235A, and P331S, or a human Igγ1 constant region having amino acid variations of L234A, L235A, P331S, and I253A. In the case where the heavy chain constant region is a human Igγ4 constant region, the human Igγ4 constant region is preferably a human Igγ4 constant region having amino acid variations of S228P and L235E.

[0039]

In the case where the anti-human Tie2 antibody of the present invention is a tandem antibody, as a linker that links the structures consisting of a heavy chain variable region and a CH1 region, any peptide (peptide linker) can be used as long as the antibody
 5 has such a function. The length of the peptide linker and the amino acid sequence can be appropriately selected by a person skilled in the art. The peptide linker preferably has 5 to 70 amino acids in length. The peptide linker preferably comprises the amino acid sequence of a hinge region or a portion thereof. The hinge region means a region that exists between the CH1 region and the CH2 region of an antibody, and examples of the
 10 hinge region to be used comprise a hinge region of IgG1 or IgG3. A portion of the hinge region means a region having at least 5 successive amino acids in the hinge region, and preferably means a region having at least 5 successive amino acids from the N terminus of the hinge region. Examples of a part of the hinge region include a region having 5 successive amino acids from the N terminal (consisting of the amino acid sequence of the
 15 amino acid numbers 1 to 5 of SEQ ID NO: 13) in the case of the hinge region of IgG1 and a region having 12 successive amino acids from the N terminal (consisting of the amino acid sequence of the amino acid numbers 1 to 12 of SEQ ID NO: 14) in the case of the hinge region of IgG3. In one embodiment, the linker comprises the amino acid sequence of a region having at least 5 successive amino acids from the N terminus of the hinge
 20 region and comprises amino acid sequence GlySer at the C terminus of the linker. Examples of such a linker comprise a peptide linker consisting of the amino acid sequence shown by any one of SEQ ID NOS: 13 to 20, and the linker preferably consists of the amino acid sequence shown by SEQ ID NO: 13.

[0040]

25 In one embodiment, the anti-human Tie2 antibody of the present invention is an anti-human Tie2 antibody having any one of the following characteristics i) to iv).

i) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

30 ii) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 6 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

iii) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 8 and four light chains consisting of the
 35 amino acid sequence shown by SEQ ID NO: 4.

iv) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 10 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0041]

It is known that when an antibody is expressed in cells, the antibody is modified after translation. Examples of the posttranslational modification include cleavage of lysine at the C terminal of the heavy chain by a carboxypeptidase; modification of
5 glutamine or glutamic acid at the N terminal of the heavy chain and the light chain to pyroglutamic acid by pyroglutamylation; glycosylation; oxidation; deamidation; and glycation, and it is known that such posttranslational modifications occur in various antibodies (Journal of Pharmaceutical Sciences, 2008, Vol. 97, p. 2426-2447).

[0042]

10 The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention includes an anti-human Tie2 antibody or an antigen-binding fragment thereof, which has undergone posttranslational modification. Examples of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention, which undergoes posttranslational modification, include anti-human Tie2 antibodies or antigen-
15 binding fragments thereof, which have undergone pyroglutamylation at the N terminal of the heavy chain variable region and/or deletion of lysine at the C terminal of the heavy chain. It is known in the field that such posttranslational modification due to pyroglutamylation at the N terminal and deletion of lysine at the C terminal does not have any influence on the activity of the antibody (Analytical Biochemistry, 2006, Vol. 348, p.
20 24-39).

[0043]

In one embodiment, the anti-human Tie2 antibody of the present invention is an anti-human Tie2 antibody having any one of the following characteristics (1) to (4).

(1) An anti-human Tie2 antibody comprising two heavy chains consisting of the
25 amino acid sequence in which glutamic acid of the amino acid number 1 of SEQ ID NO: 2 is modified to pyroglutamic acid and/or lysine of the amino acid number 679 of SEQ ID NO: 2 is deleted and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(2) An anti-human Tie2 antibody comprising two heavy chains consisting of the
30 amino acid sequence in which glutamic acid of the amino acid number 1 of SEQ ID NO: 6 is modified to pyroglutamic acid and/or lysine of the amino acid number 679 of SEQ ID NO: 6 is deleted and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(3) An anti-human Tie2 antibody comprising two heavy chains consisting of the
35 amino acid sequence in which glutamic acid of the amino acid number 1 of SEQ ID NO: 8 is modified to pyroglutamic acid and/or lysine of the amino acid number 679 of SEQ ID NO: 8 is deleted and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(4) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence in which glutamic acid of the amino acid number 1 of SEQ ID NO: 10 is modified to pyroglutamic acid and/or lysine of the amino acid number 676 of SEQ ID NO: 10 is deleted and four light chains consisting of the amino acid sequence shown by
5 SEQ ID NO: 4.

[0044]

In one embodiment, the anti-human Tie2 antibody of the present invention is an anti-human Tie2 antibody having the following characteristics.

An anti-human Tie2 antibody comprising two heavy chains consisting of the
10 amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0045]

The present invention further includes an anti-human Tie2 antibody or an antigen-binding fragment thereof, having the following characteristics.

15 An anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions,

in which the heavy chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3
20 consisting of the amino acid sequence of the amino acid numbers 99 to 111 of SEQ ID NO: 2,

the light chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3
25 consisting of the amino acid sequence of the amino acid numbers 94 to 102 of SEQ ID NO: 4, and

the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites.

30 [0046]

In addition, the present invention further includes an anti-human Tie2 antibody having the following characteristics.

An anti-human Tie2 antibody comprising two heavy chains and four light chains, in which

35 each heavy chain comprises two structures consisting of a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of

the amino acid numbers 99 to 111 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the carboxy terminus of one of the structures is linked to the amino terminus of the other structure through a linker, and

each light chain comprises a light chain variable region comprising CDR1
5 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of the amino acid numbers 94 to 102 of SEQ ID NO: 4, and a light chain constant region.

[0047]

10 The anti-human Tie2 antibody of the present invention is an antibody that binds to a human Tie2. Whether the antibody binds to the human Tie2 (Accession No. NP_000450.2) can be confirmed by using a known binding activity measurement method. Examples of the binding activity measurement method include a method of Enzyme-Linked ImmunoSorbent Assay (ELISA) or the like. In a case of using the ELISA, in an
15 exemplary method, a protein formed by fusion of the human Tie2 with a human Fc is immobilized on an ELISA plate, and a test antibody is added thereto to be reacted. A secondary antibody such as a biotin-labeled anti-IgG antibody is reacted with the resultant, washed, and then reacted with streptavidin to which an enzyme such as an alkaline phosphatase is bound. After washing, it is possible to confirm whether the test antibody
20 binds to the human Tie2 by carrying out activity measurement using an activity-detecting reagent (for example, in the case of the alkaline phosphatase, Chemiluminescent Ultra Sensitive AP Microwell and/or Membrane Substrate (450 nm) (BioFX, APU4-0100-01) or the like)). As a specific method for evaluating the activity, the same method as the one described in Example 12 as described later, for example, can be used.

25 [0048]

The anti-human Tie2 antibody of the present invention further includes an antibody binding to Tie2 derived from other animals (for example, monkey Tie2) in addition to binding to a human Tie2 as long as it is an antibody binding to a human Tie2.

[0049]

30 Preferably, the anti-human Tie2 antibody of the present invention binds to a human Tie2, and further, has anti-apoptotic activity with respect to a human Tie2-expressing cell. As a specific method for evaluating whether the antibody has anti-apoptotic activity with respect to a human Tie2-expressing cell, for example, the same method as the one described in Example 4 as described later can be used.

35 [0050]

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention includes a tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof which binds to the same human Tie2 epitope as the anti-human Tie2

antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, or as the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four
 5 light chains consisting of the amino acid sequence shown by SEQ ID NO: 4. Here, the epitope refers to an antigen site recognized by an antibody.

[0051]

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention includes a tetravalent anti-human Tie2 antibody or an antigen-binding
 10 fragment thereof, which binds to an epitope comprising at least one amino acid of the amino acids of the amino acid numbers 192, 195 and 197 of a human Tie2 (Accession No. NP_000450.2).

[0052]

Moreover, the anti-human Tie2 antibody or the antigen-binding fragment thereof
 15 of the present invention includes a tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof, which binds to an epitope comprising the amino acids of the amino acid numbers 192, 195 and 197 of a human Tie2 (Accession No. NP_000450.2).

[0053]

The tetravalent anti-human Tie2 antibody or the antigen-binding fragment thereof,
 20 which binds to the same human Tie2 epitope as the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, or as the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of
 25 the amino acid sequence shown by SEQ ID NO: 4 can be acquired by using a known method for determining an epitope. Examples of the method for determining an epitope include hydrogen/deuterium exchange mass spectrometry, X-ray crystal structure analysis, ELISA and a surface plasmon resonance phenomenon using an amino acid substitution mutant of a human Tie2, a partial peptide of human Tie2, or the like, and the like.

30 [0054]

It is possible to check whether the test antibody binds to the same human Tie2 epitope as the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, or as the anti-human Tie2 antibody
 35 comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4 by using the well-known method for determining an epitope as described above. In the case of using hydrogen/deuterium exchange mass

spectrometry, a human Tie2 with deuterium substitution in the absence of a test antibody and a human Tie2 with deuterium substitution in the presence of a test antibody are each decomposed by peptides, and the amount of molecules of each peptide is measured to calculate the ratio of deuterium substitution. The human Tie2 epitope of the test antibody
5 can be determined from the difference in the ratios of deuterium substitution of the human Tie2 according to the presence or absence of the test antibody. In the case of using ELISA, a point mutant of a human Tie2 is prepared. The mutant human Tie2 is immobilized and a test antibody is added thereto to undergo a reaction. After the reaction, a secondary antibody such as a biotin-labeled anti-human kappa light chain
10 antibody is reacted and washed. Thereafter, an alkaline phosphatase-labeled streptavidin (Thermo Fisher Scientific, 21324) is reacted therewith and washed. Further, it is possible to identify whether or not the test antibody binds to the mutant human Tie2 by carrying out activity measurement using Chemiluminescent Ultra Sensitive AP Microwell and/or Membrane Substrate (450 nm), or the like. It is possible to determine an epitope of the
15 test antibody by evaluating the binding activity to various types of mutant human Tie2. In the case where the epitope of the test antibody comprises at least one amino acid in the epitope of the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, or the anti-human Tie2 antibody
20 comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, it can be determined that the test antibody binds to the same human Tie2 epitope as the anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains
25 consisting of the amino acid sequence shown by SEQ ID NO: 4, or as the anti-human Tie2 antibody comprising a heavy chain consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and a light chain consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0055]

30 The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention can be easily prepared by a person skilled in the art, using a method known in the art, based on the sequence information of the heavy chain variable region and the light chain variable region of the antibody of the present invention, as disclosed in the present specification. The anti-human Tie2 antibody or the antigen-binding fragment
35 thereof of the present invention is not particularly limited, but can be produced in accordance with the method described in <Method for Producing Anti-Human Tie2 Antibody of the Present Invention and Anti-Human Tie2 Antibody Produced by the Method> as described later, for example.

[0056]

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is further purified as needed, and formulated according to a conventional method. It may be used for the prevention or the treatment of blood vessel-related diseases such as diabetic retinopathy, diabetic macular edema, sepsis, acute hepatic disorders, acute renal disorders, acute pulmonary disorders, systemic inflammatory reaction syndrome, peripheral arterial occlusive disease, or critical limb ischemia.

[0057]

<Polynucleotide of the Present Invention>

The polynucleotide of the present invention includes a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and a polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention.

[0058]

In one embodiment, the polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is a polynucleotide comprising a base sequence encoding the heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2.

[0059]

Examples of the polynucleotide comprising a base sequence encoding the heavy chain variable region shown by the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 include a polynucleotide comprising the base sequence of the base numbers 1 to 366 of SEQ ID NO: 1.

[0060]

In a preferred embodiment, the polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 2, a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6, a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 8, or a polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10.

[0061]

Examples of the polynucleotide comprising a base sequence encoding the heavy

chain consisting of the amino acid sequence shown by SEQ ID NO: 2 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 1. Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 6 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 5. Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 8 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 7. Examples of the polynucleotide comprising a base sequence encoding the heavy chain consisting of the amino acid sequence shown by SEQ ID NO: 10 include a polynucleotide comprising the base sequence shown by SEQ ID NO: 9.

[0062]

In one embodiment, the polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is a polynucleotide comprising a base sequence encoding the light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4.

[0063]

Examples of the polynucleotide comprising a base sequence encoding the light chain variable region shown by the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4 include a polynucleotide comprising the base sequence of the base numbers 1 to 339 of SEQ ID NO: 3.

[0064]

In a preferred embodiment, the polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention is a polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0065]

Examples of the polynucleotide comprising a base sequence encoding the light chain consisting of the amino acid sequence shown by SEQ ID NO: 4 include a polynucleotide comprising a base sequence shown by SEQ ID NO: 3.

[0066]

The polynucleotide of the present invention can be easily prepared by a person skilled in the art using a known method in the field based on the base sequence. For example, the polynucleotide of the present invention can be synthesized using a known gene synthesis method in the field. As the gene synthesis method, various methods such as a synthesis method of antibody genes described in WO90/07861 known by a person skilled in the art can be used. Further, once the polynucleotide of the present invention is

acquired, it is possible to acquire other polynucleotides of the present invention by introducing a variation into a predetermined site of the polynucleotide. As such a method for introducing the variation, various methods known to a person skilled in the art, such as a site-specific mutagenesis method (Current Protocols in Molecular Biology edit., 1987,
5 John Wiley & Sons Section 8.1-8.5), can be used.

[0067]

<Expression Vector of the Present Invention>

The expression vector of the present invention includes the polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human
10 Tie2 antibody or the antigen-binding fragment thereof of the present invention and/or the polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention. Tetravalent antibodies in various formats and methods for producing the same are well-known in the art, and the expression vector of the present invention can be easily
15 established by a person skilled in the art according to such production methods or the formats of the tetravalent antibodies to be expressed.

[0068]

Preferred examples of the expression vector of the present invention include an expression vector comprising a polynucleotide comprising a base sequence encoding the
20 heavy chain of the anti-human Tie2 antibody of the present invention, an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody of the present invention, and an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and a polynucleotide comprising a base sequence
25 encoding the light chain of the antibody.

[0069]

The expression vector used to express the polynucleotide of the present invention are not particularly limited as long as a polynucleotide comprising the base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-
30 biding fragment thereof of the present invention and/or a polynucleotide comprising the base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-biding fragment thereof of the present invention can be expressed in various host cells of eukaryotic cells (for example, animal cells, insect cells, plant cells, and yeast) and/or prokaryotic cells (for example, Escherichia coli), and the polypeptides encoded by
35 these can be produced. Examples of the expression vector include plasmid vectors, viral vectors (for example, adenovirus, adeno-associated virus, Sendai virus or retrovirus), and the like. Preferably pEE6.4 or pEE12.4 (Lonza, Inc.) can be used. Further, antibody genes can be expressed by using expression vectors comprising human Ig constant region

genes in advance such as AG- γ 1 or AG- κ (for example, see WO94/20632).

[0070]

The expression vector of the present invention may comprise a promoter that is operably linked to the polynucleotide of the present invention. Examples of the promoter for expressing the polynucleotide of the invention with animal cells include a virus-derived promoter such as CMV, RSV, or SV40, an actin promoter, an EF (elongation factor) 1 α promoter, and a heat shock promoter. Examples of promoters for expression by bacteria (for example, *Escherichia*) include a trp promoter, a lac promoter, λ PL promoter, and tac promoter. Further, examples of promoters for expression by yeast include a GAL1 promoter, a GAL10 promoter, a PH05 promoter, a PGK promoter, a GAP promoter, and an ADH promoter.

[0071]

In the case of using an animal cell, an insect cell, or yeast as the host cell, the expression vector of the present invention may comprise initiation codon and termination codon. In this case, the expression vector of the present invention may comprise an enhancer sequence, an untranslated region on the 5' side and the 3' side of genes encoding the antibody of the present invention or the heavy chain variable region or the light chain variable region, a secretory signal sequence, a splicing junction, a polyadenylation site, or a replicable unit. When *Escherichia coli* is used as the host cell, the expression vector of the present invention may comprise an initiation codon, a termination codon, a terminator region, and a replicable unit. In this case, the expression vector of the present invention may comprise a selection marker (for example, tetracycline resistant genes, ampicillin resistant genes, kanamycin resistant genes, neomycin resistant genes, or dihydrofolate reductase genes) which is generally used according to the necessity.

[0072]

<Transformed Host Cell of the Present Invention>

The transformed host cell of the present invention includes a host cell transformed with the expression vector of the present invention which is selected from the group consisting of (a) to (d) below:

- (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention, and a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof;
- (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the

light chain variable region of the antibody or the antigen-binding fragment thereof;

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention; and

5 (d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention.

[0073]

In one embodiment, the transformed host cell of the present invention is a host cell
10 transformed with the expression vector of the present invention, which is selected from the group consisting of (a) to (d) below:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and a polynucleotide comprising a base sequence encoding the light
15 chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

20 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody of
25 the present invention.

[0074]

Preferred examples of the transformed host cell of the present invention include a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present
30 invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody, and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

35 [0075]

The transformed host cell is not particularly limited as long as the host cell is appropriate for the expression vector being used, transformed with the expression vector, and can express the antibody. Examples of the transformed host cell include various cells

such as natural cells or artificially established cells which are generally used in the field of the present invention (for example, animal cells (for example, CHO-K1SV cells), insect cells (for example, Sf9), bacteria (for example, Escherichia), yeast (for example, Saccharomyces or Pichia) or the like). Preferably cultured cells such as CHO cells
 5 (CHO-K1SV cells, CHO-DG 44 cells, or the like) 293 cells, or NS0 cells can be used.

[0076]

A method of transforming the host cell is not particularly limited, but, for example, a calcium phosphate method or an electroporation method can be used.

[0077]

10 <Method for Producing Anti-Human Tie2 Antibody of the Present Invention and Anti-Human Tie2 Antibody Produced by the Method>

Examples of the method for producing the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention include a method for producing an anti-human Tie2 antibody or a antigen-binding fragment thereof, comprising culturing
 15 host cell(s) selected from the group consisting of (a) to (c) below to express a tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and a
 20 polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and an
 25 expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof; and

(c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and a host
 30 cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof.

[0078]

In one embodiment, examples of the method for producing the anti-human Tie2
 35 antibody of the present invention include a method for producing an anti-human Tie2 antibody, comprising culturing host cell(s) selected from the group consisting of (a) to (c) below to express an anti-human Tie2 antibody:

(a) a host cell transformed with an expression vector comprising a polynucleotide

comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

5 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

10 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody of the present invention and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody.

[0079]

The method for producing the anti-human Tie2 antibody of the present invention is not particularly limited as long as it comprises a step of culturing the transformed host cells
15 of the present invention to express the anti-human Tie2 antibody. Examples of the preferred host cells for use in the method include the preferred transformed host cells of the present invention as described above.

[0080]

The transformed host cell can be cultured by known methods. Culture
20 conditions, for example, the temperature, pH of culture medium, and the culture time are appropriately selected. In a case where the host cell is an animal cell, examples of the culture medium include MEM culture medium supplemented with approximately 5% to 20% of fetal bovine serum (Science, 1959, Vol. 130, No. 3373, p. 432 to 7), DMEM culture medium (Virology, 1959, Vol. 8, p. 396), and RPMI1640 culture medium (J. Am.
25 Mde. Assoc., 1967, Vol. 199, p. 519), a 199 culture medium (Exp. Biol. Med., 1950, Vol. 73, p. 1 to 8). The pH of the culture medium is preferably approximately 6 to 8, and the culture is generally carried out at approximately 30°C to 40°C for approximately 15 hours to 72 hours while air ventilating and stirring if necessary. In a case where the host cell is an insect cell, as the culture medium, for example, Grace's culture medium (Proc. Natl.
30 Acad. Sci. USA, 1985, Vol. 82, p. 8404) supplemented with fetal bovine serum can be used. The pH of the culture medium is preferably approximately 5 to 8, and the culture is generally carried out at approximately 20°C to 40°C for approximately 15 hours to 100 hours while air ventilating and stirring if necessary. In a case where the host cell is Escherichia coli or yeast, as the culture medium, for example, liquid culture medium
35 supplemented with a source of nutrients is appropriate. It is preferable that the nutrient culture medium contain a carbon source, an inorganic nitrogen source, or an organic nitrogen source necessary for the growth of the transformed host cell. Examples of the carbon source include glucose, dextran, soluble starch, and sucrose and examples of the

inorganic nitrogen source or the organic nitrogen source include ammonium salts, nitrate salts, amino acids, corn steep liquor, peptone, casein, meat extract, soybean meal, and potato extract. Other nutrients (for example, inorganic salts (for example, calcium chloride, sodium dihydrogen phosphate, and magnesium chloride), vitamins), and
5 antibiotics (for example, tetracycline, neomycin, ampicillin, and kanamycin) may be contained as desired. The pH of the culture medium is preferably approximately 5 to 8. In a case where the host cell is *Escherichia coli*, preferred examples of the culture medium include LB culture medium and M9 culture medium (Mol. Clo., Cold Spring Harbor Laboratory, Vol. 3, A2.2). The culture is generally carried out at approximately 14°C to
10 39°C for approximately 3 hours to 24 hours while air ventilating and stirring if necessary. In a case where the host cell is yeast, as the culture medium, for example, Burkholder minimal medium (Proc. Natl. Acad. Sci. USA, 1980, Vol. 77, p. 4505) can be used. The culture is generally carried out at approximately 20°C to 35°C for approximately 14 hours to 144 hours while air ventilating and stirring if necessary. By carrying out the culture in
15 the above-described manner, it is possible to express the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention.

[0081]

The method of producing the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention may comprise recovering, preferably isolating or
20 purifying the anti-human Tie2 antibody or the antigen-binding fragment thereof from the transformed host cell in addition to culturing the transformed host cell of the present invention to express the anti-human Tie2 antibody or the antigen-binding fragment thereof. Examples of the isolation or purification method include methods using solubility such as salting-out and the solvent precipitation method, methods using the difference in molecular
25 weight such as dialysis, ultrafiltration, and gel filtration, methods using an electric charge such as ion exchange chromatography and hydroxylapatite chromatography, methods using specific affinity such as affinity chromatography, methods using the difference in hydrophobicity such as reverse phase high performance liquid chromatography, and methods using the difference in the isoelectric point such as isoelectric focusing phoresis.
30 Preferably, the antibody accumulated in a culture supernatant can be purified by various chromatographies, for example, column chromatography using Protein A column or Protein G column.

[0082]

The anti-human Tie2 antibody or the antigen-binding fragment thereof of the
35 present invention also includes an anti-human Tie2 antibody or an antigen-binding fragment thereof produced by the method for producing the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention.

[0083]

<Pharmaceutical Composition of the Present Invention>

The pharmaceutical compositions of the present invention include a pharmaceutical composition comprising the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention and pharmaceutically acceptable
5 excipients. The pharmaceutical composition of the present invention can be prepared by a method being generally used with excipients being generally used in the field, that is, excipients for medicine or carriers for medicine. Examples of dosage forms of the pharmaceutical compositions include parenteral drug such as an injection drug and a drip infusion drug, and these can be administered by intravenous administration, subcutaneous
10 administration, intraocular administration, or the like. In drug preparation, excipients, carriers, and additives in accordance with the dosage forms can be used within the pharmaceutically acceptable range.

[0084]

The pharmaceutical compositions of the present invention may comprise plural
15 kinds of anti-human Tie2 antibodies or antigen-binding fragments thereof of the present invention. For example, the present invention includes a pharmaceutical composition comprising an antibody or an antigen-binding fragment thereof, which does not undergo posttranslational modification and an antibody or an antigen-binding fragment thereof derived from posttranslational modification of the antibody or the antigen-binding
20 fragment thereof.

[0085]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody or an antigen-binding fragment thereof, includes a pharmaceutical composition as described below.

25 A pharmaceutical composition comprising an anti-human Tie2 antibody or an antigen-binding fragment thereof, in which the anti-human Tie2 antibody or the antigen-binding fragment thereof comprises four heavy chain variable regions and four light chain variable regions, the heavy chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2, the light chain variable region consists of
30 the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites, and an antibody or an antigen-binding fragment thereof derived from posttranslational modification of the antibody or the antigen-binding fragment thereof.

35 [0086]

In one embodiment, the pharmaceutical composition comprising the anti-human Tie2 antibody of the present invention includes the pharmaceutical composition as described below.

A pharmaceutical composition comprising an anti-human Tie2 antibody which is an anti-human Tie2 antibody and an antibody formed by posttranslational modification of the antibody, comprising two heavy chains and four light chains, in which each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker, and each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region, and the antibody comprises four antigen-binding sites, and an antibody derived from posttranslational modification of the antibody.

[0087]

The pharmaceutical compositions of the present invention also include a pharmaceutical composition comprising an antibody in which lysine of the C terminus of the heavy chain is deleted, an antibody or an antigen-binding fragment thereof with post-translational modification to N terminal, an antibody in which lysine of the C terminus of the heavy chain is deleted and posttranslational modification to N terminal is made, and/or an antibody which has lysine in the C terminus of the heavy chain and does not have post-translational modification to N terminal.

[0088]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody includes a pharmaceutical composition comprising at least two kinds of anti-human Tie2 antibodies selected from (1) to (4) below.

(1) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(2) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of SEQ ID NO: 2 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(3) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(4) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0089]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody includes a pharmaceutical composition comprising at least two kinds of anti-human Tie2 antibodies selected from (1) to (4) below.

(1) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 6 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(2) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of SEQ ID NO: 6 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(3) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 6 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(4) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 6 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0090]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody includes a pharmaceutical composition comprising at least two kinds of anti-human Tie2 antibodies selected from (1) to (4) below.

(1) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 8 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(2) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of SEQ ID NO: 8 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(3) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 8 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(4) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 8 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0091]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody includes a pharmaceutical composition

comprising at least two kinds of anti-human Tie2 antibodies selected from (1) to (4) below.

(1) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 675 of SEQ ID NO: 10 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

5 (2) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of SEQ ID NO: 10 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

10 (3) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 675 of SEQ ID NO: 10 in which glutamic acid of amino acid number 1 is modified to pyroglutamic acid and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

(4) An anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 10 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[0092]

In one embodiment, the pharmaceutical composition of the present invention comprising an anti-human Tie2 antibody or an antigen-binding fragment thereof also includes the pharmaceutical composition as described below.

20 A pharmaceutical composition comprising an anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4, an anti-human Tie2 antibody comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains
25 consisting of the amino acid sequence shown by SEQ ID NO: 4, and a pharmaceutically acceptable excipient.

[0093]

The amount of the anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention added in formulation varies depending on the degree of
30 symptoms and the age of a patient, a dosage form of a preparation to be used, the binding titer of an antibody, or the like, and for example, an amount added of approximately 0.001 mg/kg to 100 mg/kg can be used.

[0094]

The pharmaceutical composition of the present invention can be used as an agent
35 for preventing or treating blood vessel-related diseases, for example, diabetic retinopathy, diabetic macular edema, sepsis, acute hepatic disorders, acute renal disorders, acute pulmonary disorders, systemic inflammatory reaction syndrome, peripheral arterial occlusive disease, or critical limb ischemia.

[0095]

The present invention includes a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia, comprising the anti-human Tie2 antibody of the present invention. Further, the present invention
 5 includes a method for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia, comprising administering a therapeutically effective amount of the anti-human Tie2 antibody of the present invention. Further, the present invention includes the anti-human Tie2 antibody of the present invention for use in preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia. In
 10 addition, the present invention includes use of the anti-human Tie2 antibody of the present invention for preparation of a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

[0096]

<Fusion Antibody and Modification Antibody>

15 Any person skilled in the art can prepare a fusion antibody in which an antibody or an antigen-binding fragment thereof is fused with another peptide or protein, and can also prepare a modification antibody to which a modifying agent is bound, using a known method in the field. The anti-human Tie2 antibody or the antigen-binding fragment thereof of the present invention includes the antibody and the antigen-binding fragment
 20 thereof in the form of such a fusion or a modification. For example, the anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, in which the heavy chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2, the light chain variable region consists of the amino acid sequence of the amino acid
 25 numbers 1 to 113 of SEQ ID NO: 4, the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment thereof comprises four antigen-binding sites, includes an anti-human Tie2 antibody or an antigen-binding fragment thereof fused with another peptide or protein, and an anti-human Tie2 antibody or an antigen-binding fragment thereof having a
 30 modifying agent bound thereto. The other peptide or protein for use in the fusion is not particularly limited as long as the antibody or the antigen-binding fragment thereof of the present invention as a fusion has binding activity to a human Tie2, and examples thereof include human serum albumin, various tag peptides, artificial helix motif peptides, maltose-binding protein, a glutathione S transferase, various toxins, and other peptides or
 35 proteins capable of promoting multimerization. The modifying agent for use in the modification is not particularly limited as long as the antibody or an antigen-binding fragment thereof of the present invention as a modification antibody has binding activity to a human Tie2, and examples thereof include polyethylene glycol, sugar chains,

phospholipids, liposomes, and low-molecular compounds.

[0097]

The present invention has been described and specific examples referred to for better understanding will be provided, but these are merely examples and the present invention is not limited thereto.

Examples

[0098]

With regard to parts using commercially available kits or reagents, the experiments were carried out according to the described protocol unless specifically otherwise noted. For the sake of convenience, a concentration in mol/L is represented by M. For example, a 1 M aqueous sodium oxide solution means a 1 mol/L aqueous sodium oxide solution.

[0099]

(Example 1: Preparation of Hybridoma Producing Anti-human Tie2 Antibody)

Antibody was prepared by using the "VelocImmune" (VelocImmune antibody technology: Regeneron, Inc. (US Patent No. 6596541)) - human monoclonal antibody developing technology - mouse. A recombinant human Tie2-Fc chimeric protein (R&D, 313-TI-100) was injected into the VelocImmune mouse, together with an adjuvant for causing an immune reaction, so as to perform immunization. According to an ordinary method, the lymph node of the immunized mouse was extracted, and the lymphocytes were collected and cell-fused with mouse-derived myeloma cell SP2/0 (ATCC: CRL-1581), thereby preparing a hybridoma. The hybridoma was monocloned and each clone was cultured in a CD Hybridoma Medium (Invitrogen) which is a serum-free culture medium. The antibody was purified from the obtained culture supernatant using a Protein G Column (GE Healthcare). The antibody obtained by using the VelocImmune technology is an antibody having a variable region of the human antibody and a constant region of the mouse antibody (also referred to a chimeric antibody).

[0100]

(Example 2: Cell ELISA Assay)

In order to measure the antigen-binding activity of the antibody, the bindings of the antibody to a human Tie2, a monkey Tie2, a rat Tie2, and a mouse Tie2 were each evaluated by cell ELISA assay using a human Tie2-expressing CHO cell, a monkey Tie2-expressing CHO cell, a rat Tie2-expressing CHO cell, and a mouse Tie2-expressing CHO cell.

[0101]

(Example 3: Evaluation of Competitive Activity Using Modified Ang-1)

In order to evaluate the Ang-2 competitive activity of the antibody, the inhibition of the binding of a modified Ang-1 (Proc. Natl. Acad. Sci., 2004, Vol. 101, pp. 5547-5552,

also referred to as COMP-Ang1.) to Tie2 was evaluated. The COMP-Ang1 is a modified Ang-1 in which a site not involved in the binding to Tie2 is modified, and its competitive action against Ang-2 can be evaluated by evaluating the competitive action against COMP-Ang1 from the viewpoints that the binding capacity of COMP-Ang1 to Tie2 is maintained (Proc. Natl. Acad. Sci. 2004, Vol. 101, pp. 5547-5552), and Ang-1 and Ang-2 bind to the same site of Tie2 with the same level of affinity (Science, 1997, Vol. 277, pp. 55-60).

[0102]

An expression vector of COMP-Ang1 was introduced into an HEK293 cell. The COMP-Ang1 was purified from a culture supernatant of the HEK293 cell, and biotin-labeled. The biotin-labeled COMP-Ang1 and the purified antibody obtained in Example 1 were mixed, and the mixture was added to a plate immobilized with a recombinant human Tie2-Fc chimeric protein. For the detection of the biotin-labeled COMP-Ang1 thus bound, a streptavidin-labeled HRP was used. A TMB color developing reagent (Dako, S1599) was added thereto and left to stand. Further, a 2 M sulfuric acid was then added thereto to stop the reaction and an absorbance at 450 nm was measured. In this manner, the competitive action of the antibody against the COMP-Ang1 was evaluated.

[0103]

(Example 4: Evaluation of Anti-Apoptotic Activity Using Human Tie2-Expressing BaF3 Cell)

A mouse pro-B cell strain BaF3 cell which stably expresses a human Tie2 (hereinafter also referred to as a human Tie2-expressing BaF3 cell) was prepared by introducing a plasmid containing a human Tie2 gene shown by SEQ ID NO: 21 to the cell by electroporation according to the method described in Immunity, 1998, Vol. 9, pp. 677-686. Thereafter, the anti-apoptotic activity of the antibody was evaluated using the same cell.

[0104]

The human Tie2-expressing BaF3 cell was suspended in an RPMI1640 medium (Life Technologies) supplemented with 0.05% fetal bovine serum albumin at 2×10^5 cells/mL, and distributed in the amount of 80 μ L per well in a 96-well plate for floating cells (Sumitomo Bakelite Co., Ltd., MS-8096R). Thereafter, 20 μ L of the purified antibody obtained in Example 1 or Ang-1 was added thereto. After culturing for 72 hours in a CO₂ incubator set to 37°C, 50 μ L of the cell suspension was transferred to a white 96-well plate (Nunc, 236108). According to an intracellular ATP quantification reagent CellTiter Glo Luminescent Cell Viability Kit (Promega), by adding 50 μ L of a substrate solution diluted with an attached buffer to the cell suspension, the viability of the cell was measured, thereby evaluating anti-apoptotic activity.

[0105]

From the results of Examples 2 to 4, antibodies having a binding activity to a

human Tie2, a monkey Tie2, a rat Tie2, and a mouse Tie2, a COMP-Ang1 competitive activity, and anti-apoptotic activity to a human Tie2 were found. The purified antibody solution comprising an anti-human Tie2 antibody nominated as 2-16 which will be described later exhibited substantially the same anti-apoptotic activity as Ang-1 in Example 4, but the purified antibody solution comprising the mouse anti-human Tie2 antibody 15B8 (Patent Document 1) exhibited only approximately 60% of the maximum activity of the Ang-1.

[0106]

(Example 5: Analysis of Purified Antibody Solution Using Size Exclusion

10 Chromatography and Electrophoresis)

The purified antibody solutions identified in Examples 2 to 4 above were analyzed by size exclusion chromatography. As a result, three fractions were detected from the respective purified antibody solutions. As a result of the analysis of the respective fraction solutions by electrophoresis, it was found that the respective fractions include monomers, dimers, trimers or higher-valent multimers of the antibodies, respectively.

[0107]

Next, the respective fraction solutions were evaluated regarding the anti-apoptotic activity by the method shown in Example 4. As a result, in the fractions comprising the dimers and the fractions comprising the trimers or higher-valent multimers, potent anti-apoptotic activity was recognized. On the other hand, in the fraction comprising monomers from the respective antibodies, anti-apoptotic activity was substantially unrecognized. 15B8 was also analyzed by size exclusion chromatography as described above, and as a result, fractions showing dimers or higher-valent multimers were detected, but fractions comprising monomers were substantially undetected.

25 [0108]

From the above, it was found that in any antibody identified in Examples 2 to 4, the fractions comprising the antibodies formed into dimers or higher-order multimers pertained potent anti-apoptotic activities. It is suggested that antibodies having four or higher valences have the strong anti-apoptotic activity through Tie2 activation as a dimer is a tetravalent antibody.

[0109]

(Example 6: Evaluation of Anti-Apoptotic Activity by Cross-Linking Antibody)

From the investigations in Example 5, it is considered that the valence of the anti-human Tie2 antibody adjusted to be 4 or higher is important to induce the anti-apoptotic activity through Tie2. Thus, the anti-apoptotic activity of the anti-human Tie2 antibody which was multimerized by performing cross-linking with an anti-mouse IgG antibody was evaluated. As a cell, a human Tie2-expressing BaF3 cell and a human vascular endothelial cell HUVEC that endogenously expresses a human Tie2 were used.

[0110]

The human Tie2-expressing BaF3 cell and HUVEC were cultured in an RPMI1640 medium and an EBM-2 serum-free medium (Lonza), respectively, to which an antibody solution comprising the anti-human Tie2 antibodies indentified in Examples 2 to 4 had been added. An anti-mouse IgG antibody was added thereto to cross-link the antibodies. By employing CellTiter Glo Luminescent Cell Viability Assay, the viability of the cells was measured. By measuring the viability, the anti-apoptotic activity was evaluated.

[0111]

As a result, it was found that the cross-linking antibody of the anti-human Tie2 antibody (chimeric antibody) nominated as 2-16 has a potent anti-apoptotic activity on the human Tie2.

[0112]

(Example 7: Sequencing of Bivalent Anti-Human Tie2 Antibody)

A gene encoding the heavy and light chains of the antibody was cloned from a hybridoma producing the anti-human Tie2 antibody 2-16, and sequenced.

[0113]

After sequencing the antibody, the framework region (FR) of the light and heavy chains of 2-16 was replaced with the FR of another human antibody in order to improve the physical properties and the stability of the antibody, thereby preparing a modified variable region of anti-human Tie2 antibody 2-16A2.

[0114]

A gene encoding a signal sequence (Protein Engineering, 1987, Vol. 1, No.6, pp. 499-505) and a human Igγ1 constant region gene (consisting of the base sequence of base numbers 367 to 1356 of SEQ ID NO: 11) were linked to the 5' side and the 3' side, respectively, of the heavy chain variable region gene of 2-16A2, and the heavy chain gene was inserted into a GS vector pEE6.4. Further, a gene encoding a signal sequence (Protein Engineering, 1987, Vol. 1, No.6, pp. 499-505) and a constant region gene (consisting of the base sequence of base numbers 340 to 657 of SEQ ID NO: 3) of a human κ chain were connected to the 5' side and the 3' side, respectively, of the light chain variable region gene. This light chain gene was inserted into GS vector pEE12.4. The heavy chain gene sequence and the light chain gene sequence of the prepared antibody were analyzed using a sequencer.

[0115]

The base sequence of the heavy chain of the fully human antibody of 2-16A2 (fully human 2-16A2) and the amino acid sequence encoded by the base sequence are shown by SEQ ID NOS: 11 and 12, respectively. Further, the base sequence of the light chain of the antibody and the amino acid sequence encoded by the base sequence are

shown by SEQ ID NOS: 3 and 4, respectively. The variable region of the heavy chain shown by SEQ ID NO: 12 consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 12, and the variable region of the light chain shown by SEQ ID NO: 4 consists of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4.

[0116]

By using the GS vector as described above, into which the genes of the heavy chain and the light chain of the fully human 2-16A2 had each been inserted, the antibody expression was performed by using two types of methods, that is, transient expression and stably expression. With regard to transient expression, FreeStyle 293 cells (Invitrogen) cultured in a FreeStyle 293 Expression medium (Invitrogen) at about 1,000,000 cells/mL were transfected with both expression vectors of the heavy chain and the light chain as described above using a transfection kit, 293 Fectin (Invitrogen), and cultured for 5 days. Alternatively, Expi 293 cells (Invitrogen) cultured in an Expi 293 Expression medium (Invitrogen) at about 3,000,000 cells/mL were transfected by both expression vectors of the heavy chain and the light chain as described above using a transfection kit, ExpiFectamine 293 Transfection kit (Invitrogen), and cultured for 7 days. Alternatively, CHO-K1SV cells (Lonza) cultured in a CD-CHO medium (Invitrogen) at about 10,000,000 cells/mL were transfected both expression vectors of the heavy chain and the light chain as described above using an electroporation method, and cultured for 7 days. The fully human antibody was purified from each of the culture supernatants using a Protein A column or a Protein G column (GE HealthCare). With regard to stable expression, the GS vector as described above, into which the genes of the heavy chain and the light chain of the antibody had been each inserted, was digested with restriction enzymes of NotI and PvuI, and ligated using a Ligation-Convenience Kit (NIPPONGENE) as a kit for ligation or a ligation reagent, Ligation high Ver. 2 (TOYOBO), thereby constructing a GS vector, into which both genes of the heavy chain and the light chain had been inserted. The antibody was expressed by transfection of the expression vector into the CHO-K1SV cells. The fully human antibody was purified from culture supernatant by a Protein A column, a Protein G column, or a MabSelect SuRe (GE Healthcare, 17-5438-02).

[0117]

(Example 8: Preparation of Tetravalent Anti-Human Tie2 Antibody)

A tetravalent anti-human Tie2 antibody was prepared. The tetravalent antibody prepared in the present Example includes two heavy chains and four light chains. Each heavy chain comprises two structures consisting of a heavy chain variable region and a CH1 region, and further comprises a CH2 region, and a CH3 region, in which the C terminus of one structure consisting of the heavy chain variable region and the CH1 region is linked to the N terminus of the other structure through a linker. Each light chain

comprises a light chain variable region and a light chain constant region. The format of the present tetravalent antibody is shown in Fig. 1.

[0118]

A gene encoding a tetravalent anti-human Tie2 antibody heavy chain, in which the C terminus of a structure (consisting of the amino acid sequence of the amino acid numbers 1 to 220 of SEQ ID NO: 12) consisting of the heavy chain variable region and the CH1 region of the fully human 2-16A2 was linked to the N terminus of the fully human 2-16A2 heavy chain through a linker consisting of the amino acid sequence shown by SEQ ID NO: 13, was prepared. A gene encoding a signal sequence (Protein Engineering, 1987, Vol. 1, No. 6, pp. 499-505) was linked to the 5' side of the prepared heavy chain gene, and inserted into a GS vector pEE6.4. The above heavy chain vector and the GS vector pEE12.4, into which the light chain gene of the fully human antibody 2-16A2 prepared in Example 7 had been inserted, were combined to prepare a tetravalent anti-human Tie2 antibody using the same antibody expression and purification method as described in Example 7. The tetravalent anti-human Tie2 antibody is referred to as TIE-1-Ig γ 1-WT.

[0119]

A gene encoding a tetravalent anti-human Tie2 antibody heavy chain having a constant region of the heavy chain of TIE-1-Ig γ 1-WT substituted with a human Ig γ 4 constant region (consisting of the amino acid sequence of the amino acid numbers 123 to 220 of SEQ ID NO: 10, and consisting of the amino acid sequence of the amino acid numbers 350 to 676 of SEQ ID NO: 10) with amino acid mutations of S228P and L235E, was prepared. A gene encoding a signal sequence (Protein Engineering, 1987, Vol. 1, No. 6, pp. 499-505) was linked to the 5' side of the prepared heavy chain gene and inserted into a GS vector pEE6.4. The above heavy chain vector and the GS vector pEE12.4, into which the light chain gene of the fully human 2-16A2 prepared in Example 7 had been inserted, were combined to prepare a tetravalent anti-human Tie2 antibody using the same antibody expression and purification method as described in Example 7. The tetravalent anti-human Tie2 antibody with IgG4 is referred to as TIE-1-Ig γ 4-PE.

[0120]

The base sequence of the heavy chain of TIE-1-Ig γ 1-WT and the amino acid sequence encoded by the base sequence are shown by SEQ ID NOS: 7 and 8, respectively. The base sequence of the heavy chain of TIE-1-Ig γ 4-PE and the amino acid sequence encoded by the base sequence are shown by SEQ ID NOS: 9 and 10, respectively. The light chain of both the antibodies are the same as the light chain of the fully human antibody 2-16A2, and the base sequence of the light chain and the amino acid sequence encoded by the base sequence of the antibody are shown by SEQ ID NOS: 3 and 4, respectively.

[0121]

By using the same method, a tetravalent anti-human Tie2 antibody, in which amino acid variations of L234A, L235A, and P331S had been introduced to the constant region of the heavy chain of TIE-1-Ig γ 1-WT (referred to as TIE-1-Ig γ 1-LALA), and a tetravalent
 5 anti-human Tie2 antibody, in which amino acid variations of L234A, L235A, P331S, and I253A had been introduced to the constant region of the heavy chain of TIE-1-Ig γ 1-WT (referred to as TIE-1-Ig γ 1-I253A), were prepared.

[0122]

The base sequence of the heavy chain and the amino acid sequence encoded by the
 10 base sequence of TIE-1-Ig γ 1-LALA are shown by SEQ ID NOS: 1 and 2, respectively. The base sequence of the heavy chain and the amino acid sequence encoded by the base sequence of TIE-1-Ig γ 1-I253A are shown by SEQ ID NOS: 5 and 6, respectively. The light chains of both the antibodies were the same as the light chain of the fully human 2-16A2, and the base sequence of the light chain and the amino acid sequence encoded by
 15 the base sequence of the antibody were shown by SEQ ID NOS: 3 and 4, respectively.

[0123]

The variable regions of the heavy chains of four kinds of the tetravalent anti-human Tie2 antibodies shown by SEQ ID NOS: 2, 6, 8, and 10 are common and consist of the amino acid sequence of the amino acid numbers 1 to 120 of SEQ ID NO: 2. The
 20 CDR1, CDR2, and CDR3 of the heavy chain variable regions each consist of the amino acid sequence of the amino acid numbers 31 to 35, 50 to 66, and 99 to 111 of SEQ ID NO: 2.

[0124]

The variable regions of the light chains of four kinds of the tetravalent anti-human
 25 Tie2 antibodies shown by SEQ ID NO: 4 each consist of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4. The CDR1, CDR2, and CDR3 of the light chain variable regions consist of the amino acid sequence of the amino acid numbers 24 to 39, 55 to 61, and 94 to 102 of SEQ ID NO: 4, respectively.

[0125]

30 As a result of the analysis of the amino acid modifications of the purified TIE-1-Ig γ 1-LALA, it was found that in most of the purified antibodies, deletion of lysine at the C terminus of the heavy chain occurred.

[0126]

In addition, by using the same method, tetravalent anti-human Tie2 antibodies, in
 35 which with respect to TIE-1-Ig γ 1-WT and TIE-1-Ig γ 4-PE, the linker (consisting of the amino acid sequence shown by SEQ ID NO: 13) was substituted with other linkers (four kinds of linkers consisting of the amino acid sequences shown by SEQ ID NOS: 17 to 20 with respect to TIE-1-Ig γ 1-WT, and seven kinds of linkers consisting of the amino acid

sequences shown by SEQ ID NOS: 14 to 20 with respect to TIE-1-Ig γ 4-PE), were prepared (total 11 kinds). A linker having a length of 7 amino acids (a linker consisting of the amino acid sequence shown by SEQ ID NO: 13) to a linker having a length of 64 amino acids (a linker consisting of the amino acid sequence shown by SEQ ID NO: 20) were investigated.

[0127]

As a result of the investigations on TIE-1-Ig γ 1-WT, TIE-1-Ig γ 4-PE and 11 kinds of antibodies in which the linker were substituted, it was found that all of the anti-human Tie2 antibodies had substantially the same anti-apoptotic activities in accordance with the method of Example 4.

[0128]

(Example 9: Evaluation of Anti-Apoptotic Action of Bivalent Anti-Human Tie2 Antibody and Tetravalent Anti-Human Tie2 Antibody)

From the results of Example 5, it was suggested that a tetravalent or higher-valent antibody has a potent anti-apoptotic activity through a human Tie2 activation. Thus, the efficacy of the bivalent anti-human Tie2 antibody was compared with that of the tetravalent anti-human Tie2 antibody by measuring the anti-apoptotic action on the human Tie2-expressing BaF3 cell as an index.

[0129]

According to the method of Example 4, the anti-apoptotic action of the fully human 2-16A2 which is a bivalent antibody and TIE-1-Ig γ 1-WT which is a tetravalent antibody was evaluated by using the human Tie2-expressing BaF3 cell. The fully human 2-16A2 and TIE-1-Ig γ 1-WT which were tested antibodies were purified by MabSelect SuRe and fractionized into monomer fractions by size exclusion chromatography, thereby acquiring monomer purities of 99.98% and 99.74%, respectively. The respective antibodies were diluted with phosphate buffer saline (PBS) to from 5 ng/mL to 5000 ng/mL at an about 3-fold common ratio through seven steps, and added in the amount of 20 μ L per well. As a control, PBS, or Ang-1 diluted with PBS (R&D, 923-AN-025/CF, a final concentration of 1 ng/mL to 1000 ng/mL, diluted at an about 3-fold common ratio through 7 steps) had been added instead of the test antibodies, were prepared, respectively. For calculation of the anti-apoptotic activity at each of the concentrations of the test antibodies, the measured value of the well to which PBS had been added instead of the test antibody was set to 0%, and the average value of the measured values of the wells to which Ang-1 had been added at the concentration of 300 ng/mL and 1000 ng/mL, respectively, instead of the test antibodies, was set to 100%. The EC₅₀ value of the test antibody was calculated by analyzing the calculated anti-apoptotic activity using Sigmoid-Emax model non-linear regression analysis.

Table 1: Anti-Apoptotic Activities of Bivalent Anti-Human Tie2 Antibody and

Tetravalent Anti-Human Tie2 Antibody

[Table 1]

	EC ₅₀ value	Maximum activity of anti-apoptotic activities
TIE-1-IgY1-WT	7.10 ng/mL	104%
Fully human 2-16A2	37.6 ng/mL	22%

[0130]

5 As a result, it was found that TIE-1-IgY1-WT which is a tetravalent antibody has potent anti-apoptotic action. From the above, it was found that the tetravalent antibody has a superior anti-apoptotic activity, as compared with the bivalent antibody.

[0131]

10 (Example 10: Evaluation of Vascular Permeability Inhibitory Action of Bivalent Anti-Human Tie2 Antibody and Tetravalent Anti-Human Tie2 Antibody in Rat)

A mustard oil-induced vascular permeability model is a model with a modification applied to a Miles assay (J. Physiol., 1952, Vol. 118, pp. 228-257) which has been widely used as a plasma leakage evaluation system, and it has been reported that Ang-1 inhibits the vascular hyperpermeability in the present model (Nature Medicine, 2000, Vol. 6, pp. 15 460-463). Accordingly, in order to compare the vascular permeability inhibitory action of the bivalent anti-human Tie2 antibody with that of the tetravalent anti-human Tie2 antibody, the fully human 2-16A2 and TIE-1-IgY1-WT were evaluated using the present model.

[0132]

20 The fully human 2-16A2 or TIE-1-IgY1-WT diluted with PBS was subcutaneously administered to an SD rat (Male, 4-5-week-old, Charles River Laboratories Japan, Inc.). The treated groups were set as follows.

[Treated group (6 rats per group)]

Vehicle group:

25 Group to which PBS instead of the antibody was administered

Fully human 2-16A2 administration group:

Group to which the fully human 2-16A2 was administered (0.3 mg/kg)

TIE-1-IgY1-WT administration group:

Group to which TIE-1-IgY1-WT was administered (0.3 mg/kg)

30 [0133]

At 48 hours after the administration of the antibody, an Evans Blue dye dissolved in physiological saline (45 mg/kg, Sigma-Aldrich Corporation, E2129) was intravenously administered, immediately allyl isothiocyanate (also referred to as a mustard oil, Nacalai Tesque, Inc., 01415-92) diluted with a mineral oil (Sigma-Aldrich Corporation, M8410) of

5% was applied onto one ear, while the mineral oil was applied onto the contralateral ear, in the amount of 20 μ L. After 30 minutes, both of the ears were sampled, weighed, then immersed in 1 mL of formamide, and incubated at 70°C overnight to extract the Evans Blue dye in the ear tissue. The Evans Blue dye concentration was determined from the absorbance (a measurement wavelength of 620 nm and a control wavelength of 740 nm) of the extract to calculate the amount of the Evans Blue dye in the extract. Thereafter, by dividing the amount of the Evans Blue dye by the weight of the ear, the dye leakage amount per weight of the ear was calculated. A value obtained by subtracting the leakage amount of the Evans Blue dye of the ear having the mineral oil applied thereon from the leakage amount of the Evans Blue dye of the ear having the mustard oil applied thereon in the same individual was calculated as a final leakage amount of the Evans Blue dye of each individual. The leakage amount of the Evans Blue dye was used as an index of vascular permeability. The results are shown in Fig. 2.

[0134]

The mean value and the standard error of each group were determined. A Student t-test was used to determine a significant difference between the vehicle group and each group to which an antibody had been administrated. A case with $p < 0.05$ was intended to indicate that there was a significant difference.

[0135]

As shown in Fig. 2, compared with a vehicle group, the fully human 2-16A2 which is a bivalent antibody did not inhibit the dye leakage, whereas TIE-1-IgY1-WT which is a tetravalent antibody significantly inhibited the dye leakage. It was found that TIE-1-IgY1-WT which is a tetravalent antibody inhibited vascular hyperpermeability. From above, it was found that the tetravalent antibody has a superior vascular hyperpermeability inhibitory action, as compared with the bivalent antibody.

[0136]

From the results of Examples 9 and 10, it was found that the tetravalent anti-Tie2 antibody strongly induced an action through Tie2.

[0137]

(Example 11: Evaluation of Anti-Apoptotic Action of Tetravalent Anti-Human Tie2 Antibody (2))

For TIE-1-IgY1-LALA and TIE-1-IgY1-I253A, according to the method of Example 9, the anti-apoptotic activity of the antibody on the human Tie2-expressing BaF3 cell was evaluated. In the same concentration range as in Example 9, evaluation of each tetravalent anti-human Tie2 antibody was carried out. In this regard, when the average value of the measured values of the wells, to which each of 100 ng/mL, 300 ng/mL, and 1000 ng/mL of Ang-1 had been added, was taken as 100%, the EC_{50} value and the maximum activity of the anti-apoptotic activity of each antibody were evaluated.

Table 2: Anti-Apoptotic Activity of Each Tetravalent Anti-Human Tie2 Antibody
[Table 2]

	EC ₅₀ value	Maximum activity of anti-apoptotic activities
TIE-1-Ig γ 1-LALA	3.65 ng/mL	88%
TIE-1-Ig γ 1-I253A	5.06 ng/mL	94%

[0138]

5 As a result, it was found that both the TIE-1-Ig γ 1-LALA and the TIE-1-Ig γ 1-I253A exhibited substantially equivalent anti-apoptotic activity as Ang-1.

[0139]

(Reference Example 1: Evaluation of Anti-Apoptotic Action of 15B8)

10 For 15B8, according to the method of Example 9, the anti-apoptotic activity on the human Tie2-expressing BaF3 cell was evaluated. Evaluation of 15B8 (Patent Document 1) was carried out in the same antibody concentration range as in Example 9. Evaluation of Ang-2 (R&D, 623-AN-025) was carried out in the same manner as that for Ang-1. In this regard, when the average value of the measured values of the wells, to which 1000 ng/mL of Ang-1 had been added, was taken as 100%, the EC₅₀ value and the maximum activity of the anti-apoptotic activity were evaluated.

Table 3: Anti-Apoptotic Activity of 15B8

[Table 3]

	EC ₅₀ value	Maximum activity of anti-apoptotic activities
15B8	26.6 ng/mL	64%
Ang-2	39.3 ng/mL	67%

[0140]

20 As a result, it was found that the anti-apoptotic activity of 15B8 was about 64% of Ang-1 and had substantially equivalent anti-apoptotic activity as Ang-2.

[0141]

25 As combined with the results of Example 11, it was found that TIE-1-Ig γ 1-LALA exhibited substantially equivalent anti-apoptotic activity as Ang-1, whereas 15B8 exhibited substantially equivalent partial anti-apoptotic activity as Ang-2.

[0142]

(Example 12: Evaluation of Binding Activity of TIE-1-Ig γ 1-LALA to Tie2)

30 For TIE-1-Ig γ 1-LALA, the binding activities to each species Tie2 proteins were evaluated. A recombinant human Tie2-Fc chimeric protein (R&D, 313-TI-100), a recombinant monkey Tie2-Fc chimeric protein (Sino Biological Inc., 90292-C02H), a recombinant rat Tie2-Fc chimeric protein (R&D, 3874-T2-100), or a recombinant mouse

Tie2-Fc chimeric protein (R&D, 762-T2-100) was prepared in PBS at 1 µg/mL, added to a white Maxisorp 384-well plate (Nunc, 460372) in the amount of 20 µL per well, and incubated at 4°C overnight to perform immobilization. The next day, the immobilized solution was removed, and 20% Blocking One (Nacalai Tesque Inc., 03953-95)-containing Tris Buffer Saline (TBS) - 0.05% Tween (Wako, 310-7375) (hereinafter referred to as a TBS-T solution) was added thereto in the amount of 50 µL per well, and left to stand at room temperature for 1 hour. TIE-1-IgY1-LALA as a test antibody was diluted with a TBS-T solution containing 5% Blocking One from 0.03 ng/mL to 100 ng/mL at an about 3-fold common ratio through 8 steps, and added in the amount of 20 µL per well. As a control, a well to which a TBS-T solution had been added instead of the test antibody was prepared. The resultant was incubated at room temperature for 1.5 hours, and then washed with a TBS-T solution. As a secondary antibody, a biotin-labeled anti-human kappa light chain antibody (Immuno-Biological Laboratories Co., Ltd., 17249), which had been diluted to 0.1 µg/mL with a TBS-T solution containing 5% Blocking One, was added thereto in the amount of 20 µL per well. The resultant was incubated at room temperature for 1 hour and then washed with a TBS-T solution, and alkaline phosphatase-labeled streptavidin (Thermo Fisher Scientific Inc., 21324), which had been diluted to 0.1 µg/mL with 5% Blocking One-containing TBS-T solution, was added thereto in the amount of 20 µL per well. The resultant was incubated at room temperature for 1 hour and then washed with a TBS-T solution, and Chemiluminescent Ultra Sensitive AP Microwell and/or Membrane Substrate (450 nm) (BioFX, APU4-0100-01), which had been 5-fold diluted with 1 mM MgCl₂-containing 20 mM TBS (pH 9.8) as a substrate, was added thereto in the amount of 20 µL. The resultant was incubated at room temperature for 30 minutes, and then the chemiluminescence thereof was measured by an EnVision™ multi-label counter (PerkinElmer, Inc.). The EC₅₀ value of the test antibody was calculated by analyzing the calculated binding activity using Sigmoid-Emax model non-linear regression.

Table 4: Binding Activity of TIE-1-IgY1-LALA

[Table 4]

	EC ₅₀ value (ng/mL)			
	Human	Monkey	Rat	Mouse
TIE-1-IgY1-LALA	0.565	0.545	0.633	0.696

[0143]

As a result, it was found that TIE-1-IgY1-LALA has substantially the same high binding activity as a human Tie2, a monkey Tie2, a rat Tie2, and a mouse Tie2.

[0144]

(Reference Example 2: Evaluation of Binding Activity of 15B8 to Tie2)

According to the method of Examples 12, the binding activities of 15B8 to each

species Tie2 proteins were evaluated. In this regard, the absorbance at 450 nm was measured using an HRP-labeled anti-mouse kappa light chain antibody (SouthernBiotech, 1050-05) as a second antibody, a TMB color development reagent as a substrate, and an ARVO multi-label reader (PerkinElmer Inc.) as a measuring apparatus. In addition, 15B8 antibody concentration was adjusted to be from 1000 ng/mL to 0.3 ng/mL at an about 3-fold common ratio (diluted through eight steps), as a test antibody. The EC₅₀ value of the test antibody was calculated by analyzing the calculated binding activity using Sigmoid-Emax model non-linear regression (Table 5).

Table 5: Binding Activity of 15B8

[Table 5]

	EC ₅₀ value (ng/mL)			
	Human	Monkey	Rat	Mouse
15B8	218.7	224.3	> 1000	> 1000

[0145]

As a result, it was observed that 15B8 had binding activity to a human Tie2 and a monkey Tie2, but it was found that 15B8 has low binding activity to a rat Tie2 and a mouse Tie2.

[0146]

From the results of Example 12, it was observed that TIE-1-Ig γ 1-LALA had high binding activity to a human Tie2, a monkey Tie2, a rat Tie2, and a mouse Tie2 without a species difference therein. On the other hand, it was observed that 15B8 had a species difference in the binding activity. From the above, it was suggested that the human Tie2 epitope of TIE-1-Ig γ 1-LALA was different from the epitope of 15B8.

[0147]

(Example 13: Evaluation of Vascular Permeability Inhibitory Action of TIE-1-Ig γ 1-LALA in Rat)

According to the method of Example 10, the vascular permeability inhibitory action of TIE-1-Ig γ 1-LALA in rats was evaluated. In this regard, TIE-1-Ig γ 1-LALA was used as a test antibody, and the antibody dose was adjusted to be 0.1 mg/kg and 0.3 mg/kg. The results are shown in Fig. 3.

[0148]

The mean value and the standard error of each group were determined. A Dunnett multiple comparison test was employed to determine a significant difference between the vehicle group and each group to which the antibody had been administrated. A case in which $p < 0.05$ was intended to indicate that there was a significant difference.

[0149]

As shown in Fig. 3, compared to the vehicle group, TIE-1-Ig γ 1-LALA

significantly inhibited the dye leakage. From the above, it was found that TIE-1-Ig γ 1-LALA inhibited the vascular hyperpermeability.

[0150]

(Example 14: Retinal Edema Inhibitory Action in Mouse with Loss of Pericytes)

5 In the retinal blood vessels of a patient with diabetic retinopathy, the loss of pericytes is one of characteristic lesions (Retina, 2013, Fifth edition, pp. 925-939). Although rat models with Streptozotocin-induced diabetes are widely used on diabetic retinopathy studies, there is a limitation in the usefulness of the models in the following aspects: a period of several months is taken until the loss of pericytes is observed, retinal
10 microaneurysm which is thought to be caused by the loss of pericytes is not observed, the ratio of the pericytes to the endothelial cells is different from that of a human (Retina, 2013, Fifth edition, pp. 925-939), and apparent retinal edema is not observed (Diabetes Metab. J., 2013, Vol. 37, pp.217-224). On the other hand, in a mouse having the retinal blood vessels with the loss of pericytes by administration of an anti-PDGF receptor β
15 (PDGFR β) antibody, the lesions similar to those seen in diabetic retinopathy and diabetic macular edema, such as expansion of retinal blood vessel, retinal edema, and bleeding are observed, suggesting that the blood vessels are weakened like diabetic retinopathy and diabetic macular edema due to the loss of pericytes, although hyperglycemia is not observed (J. Clin. Invest., 2002, Vol. 110, pp. 1619-1628). Therefore, evaluation of the
20 inhibitory action on retinal edema using a model with a condition showing the loss of pericytes, which is a characteristic lesion in a patient with diabetic retinopathy, is useful to evaluate the effectiveness on diabetic retinopathy and diabetic macular edema.

[0151]

The retinal edema induced by loss of pericytes was prepared with a slight
25 modification to the method reported in J. Clin. Invest., 2002, Vol. 110, pp. 1619-1628. That is, anti-PDGFR β monoclonal antibody 1B3 (WO 2008/130704) diluted with PBS was subcutaneously administered at 25 mg/kg to C57BL/6J mouse (Charles River Laboratories Japan, Inc.) on the 2nd day after birth to induce the loss of pericytes in the retinal blood vessels.

30 [Treated Group]

Control Group (also referred to as Cont. group): 17 mice

Group to which an anti-PDGFR β antibody was not administered and PBS was administered

Vehicle group (also referred to as Veh. group): 24 mice

35 Group to which an anti-PDGFR β antibody was administered and PBS was administered, instead of TIE-1-Ig γ 1-LALA

TIE-1-Ig γ 1-LALA Group (0.1 mg/kg, 0.3 mg/kg, and 1 mg/kg): each 23 mice, 21 mice, and 21 mice

Group to which an anti-PDGFR β antibody was administered and each dose of TIE-1-IgY1-LALA was administered

[0152]

At 90 minutes before administration of the anti-PDGFR β antibody, TIE-1-IgY1-LALA diluted with PBS was subcutaneously administered at 0.1 mg/kg, 0.3 mg/kg and 1 mg/kg. At 1 week after administration of the antibody, retinal edema was evaluated. Specifically, the eyeball was extracted and fixed with 1% glutaraldehyde and 2.5% formalin containing solutions, and then a paraffin-embedded slice graft was prepared. Hematoxylin-eosin stained specimens were scanned to convert image data using a virtual slide scanner (NanoZoomer XR, Hamamatsu Photonics K. K.). In this model, retinal edema in the retinal nerve fiber layer (NFL) is reported (J. Clin. Invest., 2002, Vol. 110, pp. 1619-1628), thereby quantification of retinal edema was carried out by measuring the areas of NFL and adjacent retinal ganglion cell layer with an NPD view 2 (Hamamatsu Photonics K. K.). The results are shown in Fig. 4.

[0153]

The mean value and the standard error of each group were determined. A Dunnett multiple comparison test was employed as an assay for determining a significant difference between the vehicle group and each group to which TIE-1-IgY1-LALA had been administered. A Student t-test was used as an assay for determining a significant difference between the Cont. group and the Veh. group. A case in which $p < 0.05$ was intended to indicate that there was a significant difference in each case.

[0154]

As shown in Fig. 4, it was found that the TIE-1-IgY1-LALA group (1 mg/kg) significantly inhibited the retinal edema having retinal blood vessels with the loss of pericytes as compared with the vehicle group. From the viewpoint that TIE-1-IgY1-LALA inhibited the retinal edema caused by the retinal blood vessels with the loss of pericytes, it was suggested that TIE-1-IgY1-LALA is effective on diabetic macular edema and diabetic retinopathy.

[0155]

(Example 15: Ischemia Limb Blood Flow Improving Action in Mouse with Hindlimb Ischemia)

The model with hindlimb ischemia is a model having ischemia in the hindlimb tissue induced by ligation and excision of the blood vessel in the hindlimb on one side, and is also a representative model for evaluating the improving the ischemia symptoms (J. Vasc. Surg., 2012, Vol. 56, pp.1669-1679).

[0156]

The inguinal region of the femoral artery and vein and the saphenous artery and vein on the left hindlimb were ligated in a 10-week C57BL/6J mouse (CLEA Japan, Inc.).

Further, after the branch vessel therebetween was ligated, and the blood vessel between the ligated points was excised. Surgery was carried out under anesthesia with pentobarbital sodium (60 mg/kg, Tokyo Chemical Industry Co., Ltd.). At one week after excision of the vessel, the blood flow in the hindlimb was measured by using a laser Doppler perfusion imager MoorLDI2 (Moor Instruments Inc.) under anesthesia with pentobarbital. After confirming a decrease in the blood flow in the limb to be treated, the treated group was set as follows.

[Treated Group (10 mice per group)]

Control group:

Group to which PBS was administered instead of an antibody

TIE-1-Ig γ 1-LALA Group (1 mg/kg):

Group to which TIE-1-Ig γ 1-LALA was administered

[0157]

TIE-1-Ig γ 1-LALA diluted with PBS was subcutaneously administered at 1 mg/kg, and the amount of skin blood flow of the normal limb and the ischemic limb at one week after administration of the antibody were measured. Specifically, pentobarbital sodium (60 mg/kg) was intraperitoneally administered, followed by placing on a heating plate, so as to measure the skin blood flow of the foot at 15 minutes after administration of anesthesia. The results of the blood flow measured by taking the bottom part of the foot as a region of interest (ROI), are shown in Fig. 5.

[0158]

The mean value and the standard error of each group were determined. A Student t-test was used to determine a significant difference between the control group and the TIE-1-Ig γ 1-LALA group. A case in which $p < 0.05$ is intended to indicate that there was a significant difference.

[0159]

As shown in Fig. 5, it was found that compared with the control group, the TIE-1-Ig γ 1-LALA group significantly improved the amount of blood flow of the normal limb and the ischemic limb. Accordingly, the effectiveness of TIE-1-Ig γ 1-LALA on peripheral arterial diseases such as critical limb ischemia was suggested.

[0160]

(Example 16: Evaluation of Epitope of TIE-1-Ig γ 1-LALA: Hydrogen Deuterium Exchange Mass Spectrometry)

In order to identify the recognition epitope of TIE-1-Ig γ 1-LALA, Fab of the fully human 2-16A2 in Example 7 (hereinafter referred to as fully human 2-16A2-Fab) was prepared. Since the fully human 2-16A2-Fab has the same variable region as TIE-1-Ig γ 1-LALA, these antibodies recognize the same epitope. As an antigen, a human Tie2 protein consisting of the amino acid numbers 1 to 452 of Accession No. NP_000450.2 (hereinafter

referred to as a human Tie2 (1-452)) was prepared. The amino acid sequence is the same site used when the Tie2 binding site of Ang-2 was indentified (Nat. Struct. Mol. Biol., Vol. 13, pp. 524-532).

[0161]

5 Specifically, the fully human 2-16A2-Fab was prepared by combining a GS vector pEE6.4 in which a heavy chain gene encoding a structure (consisting of the amino acid sequence of the amino acid numbers 1 to 221 of SEQ ID NO: 12) consisting of the heavy chain variable region and the CH1 region of the fully human 2-16A2 was inserted, and the GS vector pEE12.4 in which a light chain gene of the fully human 2-16A2 was inserted,
10 and using the same method as the expression method and the purification method for the antibody described in Example 7.

[0162]

In order to obtain human Tie2 (1-452), first, human Tie2 (1-452) obtained by fusing human Fc (consisting of the amino acid sequence shown by SEQ ID NO: 23) with a
15 thrombin recognition sequence (consisting of the amino acid sequence shown by SEQ ID NO: 22) as a linker (hereinafter referred to as a human Tie2 (1-452)-Fc chimeric protein) was prepared. Specifically, by inserting a gene encoding the human Tie2 (1-452)-Fc chimeric protein into a GS vector pEE12.4, and using the same expression method and the purification method described in Example 7, the human Tie2 (1-452)-Fc chimeric protein
20 was prepared. Next, the prepared human Tie2 (1-452)-Fc chimeric protein was incubated with thrombin (GE Healthcare, 27-0846-01) at 22°C for 16 hours to cut the Fc portion, and thrombin and human Fc were removed by Benzamidine Sepharose 4 Fast Flow (high sub) (GE Healthcare) and MabSelect SuRe, thereby preparing a human Tie2 (1-452).

[0163]

25 For the purpose of indentifying the epitope site, hydrogen/deuterium exchange mass spectrometry (hereinafter referred to as H/D exchange mass spectrometry, Anal. Bioanal. Chem., 2010, Vol. 397, pp. 967-979) was carried out by using NanoAQUITY UPLC HDX Systems (Waters).

[0164]

30 Specifically, the fully human 2-16A2-Fab and human Tie2 (1-452) mixed liquid (final concentration of 50 μ M and 25 μ M, respectively) was prepared using a 20 mM citric acid buffer (pH 6) containing 120 mM sodium chloride, and incubated at 37°C overnight. As a control, a solution with only human Tie2 (1-452) was prepared using 20 mM citric acid buffer (pH 6) containing 120 mM sodium chloride. Thereafter, the solution was
35 added to a PBS buffer solution prepared using deuterium water (Kanto Chemical Co., Inc.), and incubated for 20 seconds, 1 minute, 10 minutes, 60 minutes, and 120 minutes, respectively, and deuteration was carried out. Then, an aqueous solution (pH 2.5) containing 100 mM dithiothreitol (Nacalai Tesque) and 4 M guanidine hydrochloride

(Wako Pure Chemical Industries, Ltd.) was added thereto at 0°C, and then digestion was carried out using a Pepsin Column (Prozyme (registered trademark) Immobilized Pepsin Cartridge, Applied Biosystems), and the peptide digested with a trap column (ACQUITY UPLC BEH C18 1.7 µm VanGuard Pre-Column, Waters) was captured. Then, separation
 5 was carried out by reverse phase chromatography using C18 column (ACQUITY UPLC BEH C18 1.7 µm, Waters) and the molecular weight was measured with a mass spectrometer (SynaptG2-Si, Waters). The centroid value of the isotopic distribution of all the detected peptides was calculated, and compared with centroid value of the isotopic distribution of only human Tie2 (1-452) which had undergone deuterium exchange, and the
 10 change amount with occurrence of deuterium substitution was calculated in terms of each deuteration period.

[0165]

As a result of the H/D exchange mass spectrometry, it was demonstrated the peptides of the amino acid numbers 27 to 37, 29 to 37, 29 to 38, 43 to 60, 82 to 100, 98 to
 15 107, 111 to 124, 116 to 125, 116 to 129, 119 to 129, 189 to 198 and 190 to 198 of Accession No. NP_000450.2 have inhibited deuteration in the coexistence of the antibody. The redundant domains of these peptides are arranged, further, the information of the peptides having not inhibited deuteration was added thereof and taking into consideration that two amino acids on the N-terminal side easily undergo reverse change (Proteins, 1993,
 20 Vol. 17, 75-86), five regions having inhibited deuterium substitution, that is, amino acid numbers 29 to 38, 84 to 102, 113 to 120, 126 to 129, and 191 to 198 of Accession No. NP_000450.2 as the epitope candidate sites were found. Further, as a result of the H/D exchange mass spectrometry, it was found that in the case where TIE-1-Igγ1-LALA interacts with a region consisting of these five amino acid segments or where a change in
 25 the steric structure or an allosteric effect by the antibody binding occurs, these residues are protected from hydrogen/deuterium exchange.

[0166]

(Example 17: Evaluation of Epitope of TIE-1-Igγ1-LALA: Surface Plasmon Resonance Analysis and ELISA)

30 An epitope candidate for human Tie2 of TIE-1-Igγ1-LALA was identified from H/D exchange mass spectrometry of Example 16. In order to predict the epitope portion in detail, amino acid mutants of the human Tie2 (1-452)-Fc chimeric protein were prepared, and the binding activity was evaluated using surface plasmon resonance analysis (SPR analysis) and ELISA.

35 [0167]

Based on the result of the H/D exchange mass spectrometry and the report of a region in which Ang-1 and Ang-2 bind to Tie2 (Nat. Struct. Mol. Biol., 2006, Vol. 13, pp. 524-532. Proc. Natl. Acad. Sci. USA, 2013, Vol. 110, 7205-7210), 23 amino acid mutant

proteins in which one to four amino acids were substituted with alanine (in one case, glutamic acid) of the human Tie2 (1-452) in the human Tie2 (1-452)-Fc chimeric protein as amino acid mutant proteins of the human Tie2 (1-452) were prepared (Table 6). Various mutants were prepared by the same preparation method for the human Tie2 (1-452)-Fc chimeric protein prepared in Example 16.

Table 6: Mutant human Tie2 (1-452)-Fc chimeric proteins

[Table 6]

Name of Mutant	Amino acid variation site
Human Tie2 (1-452)r1-Fc	R167A, H168A, E169A
Human Tie2 (1-452)r2-Fc	D172A, I173A
Human Tie2 (1-452)r3-Fc	R167A
Human Tie2 (1-452)r4-Fc	H168A
Human Tie2 (1-452)r5-Fc	E169A
Human Tie2 (1-452)r6-Fc	D172A
Human Tie2 (1-452)r7-Fc	I173A
Human Tie2 (1-452)g1-Fc	I194A, N197A, L198A
Human Tie2 (1-452)g2-Fc	R192A
Human Tie2 (1-452)g3-Fc	I194A
Human Tie2 (1-452)g4-Fc	G195E
Human Tie2 (1-452)g5-Fc	N197A
Human Tie2 (1-452)g6-Fc	L198A
Human Tie2 (1-452)m1-Fc	W82A, K84A
Human Tie2 (1-452)m3-Fc	S94A, K95A
Human Tie2 (1-452)y1-Fc	D37A
Human Tie2 (1-452)c1-Fc	R50A, H52A, E53A, P54A
Human Tie2 (1-452)A1-Fc	E151A
Human Tie2 (1-452)A2-Fc	V154A
Human Tie2 (1-452)A3-Fc	Y156A
Human Tie2 (1-452)A4-Fc	F161A
Human Tie2 (1-452)A5-Fc	S164A
Human Tie2 (1-452)A6-Fc	P166A

[0168]

10 SPR analysis was carried out in order to evaluate the binding activity of the human Tie2 (1-452)-Fc chimera protein and 23 mutant proteins thereof to the fully human 2-16A2-Fab.

[0169]

For SPR analysis, Biacore T200 (GE Healthcare) was used. An anti-human IgG

(Fc) antibody (Human Antibody Capture Kit, GE Healthcare) was fixed onto a CM5 sensor chip. The human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof, diluted with HBS-EP (GE Healthcare) at 5 µg/mL, were each allowed for immobilization, and the capture-amount was measured. Thereafter, the fully human 2-16A2-Fab diluted with HBS-EP to 50 nM, the binding amount thereof to the human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof were measured. Further, by dividing the binding amount with the capture-amount, the binding amount of the antibody in the unit immobilized antigen (hereinafter referred to as a binding ratio) was calculated. The arithmetic mean of three experiments and the relative value of the binding ratio of each mutant proteins when the binding ratio of the human Tie2 (1-452)-Fc chimeric protein was taken as 100% are shown in Table 7. Further, the representative measurement data is shown in Fig. 6. The method for relative comparison of the binding amounts in Biacore is described in, for example, Analytical Biochemistry, 2003, Vol. 312, pp. 113-124.

[0170]

As a result, it was found that the binding of the fully human 2-16A2-Fab was decreased in the human Tie2 (1-452)g1-Fc, the human Tie2 (1-452)g2-Fc, the human Tie2 (1-452)g3-Fc, the human Tie2 (1-452)g4-Fc, the human Tie2 (1-452)g5-Fc, the human Tie2 (1-452)m3-Fc, the human Tie2 (1-452)A1-Fc, the human Tie2 (1-452)A2-Fc, the human Tie2 (1-452)A3-Fc and the human Tie2 (1-452)A4-Fc, compared with the human Tie2 (1-452)-Fc chimeric protein.

Table 7: Results of SPR Analysis

[Table 7]

	Binding ratio	Relative value (%)
Human Tie2 (1-452)-Fc chimeric protein	0.29	100
Human Tie2 (1-452)r1-Fc	0.29	102
Human Tie2 (1-452)r2-Fc	0.28	98
Human Tie2 (1-452)r3-Fc	0.34	119
Human Tie2 (1-452)r4-Fc	0.33	113
Human Tie2 (1-452)r5-Fc	0.29	99
Human Tie2 (1-452)r6-Fc	0.29	100
Human Tie2 (1-452)r7-Fc	0.30	106
Human Tie2 (1-452)g1-Fc	0.00	0
Human Tie2 (1-452)g2-Fc	0.03	9
Human Tie2 (1-452)g3-Fc	0.06	23
Human Tie2 (1-452)g4-Fc	0.02	7
Human Tie2 (1-452)g5-Fc	0.03	9
Human Tie2 (1-452)g6-Fc	0.38	131
Human Tie2 (1-452)m1-Fc	0.31	107
Human Tie2 (1-452)m3-Fc	0.04	12
Human Tie2 (1-452)y1-Fc	0.25	87
Human Tie2 (1-452)c1-Fc	0.54	189
Human Tie2 (1-452)A1-Fc	0.10	34
Human Tie2 (1-452)A2-Fc	0.08	29
Human Tie2 (1-452)A3-Fc	0.17	59
Human Tie2 (1-452)A4-Fc	0.13	44
Human Tie2 (1-452)A5-Fc	0.39	135
Human Tie2 (1-452)A6-Fc	0.31	109

[0171]

- 5 ELSA was carried out by the method as in Example 12 in order to evaluate the binding activity of TIE-1-Igyl-LALA to the human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof.

[0172]

- 10 The human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof were diluted with PBS to 1 µg/mL, added to a white Maxisorp 384-well plate in the amount of 20 µL per well, and incubated at 4°C overnight to perform immobilization. The next day, the immobilized solution was removed, and the plate was washed with a TBS-T solution,

and incubated for 60 minutes by the addition of 50 μ L of a BlockerTM Casein in TBS (Thermo Fisher Scientific Inc., 37532) to perform blocking. The resultant was washed with a TBS-T solution, and TIE-1-Ig γ 1-LALA, diluted with 0.05% Tween 20 (Nacalai Tesque Inc., 28353-85)-containing BlockerTM Casein in TBS from 0.03 ng/mL to 100 ng/mL through eight steps, was added thereto in the amount of 20 μ L per well. The resultant was incubated at room temperature for 90 minutes and then washed with a TBS-T solution three times, and 20 μ L of a biotin-labeled anti-human kappa light chain antibody, which had been diluted to 0.1 μ g/mL with 0.05% Tween 20-containing BlockerTM Casein in TBS, was added thereto. The resultant was incubated at room temperature for 60 minutes and then washed with a TBS-T solution three times, and 20 μ L of alkaline phosphatase-labeled streptavidin, which had been diluted to 0.1 μ g/mL with 0.05% Tween 20-containing BlockerTM Casein in TBS, was added thereto. The resultant was incubated at room temperature for 60 minutes and then washed with a TBS-T solution three times, and 50 μ L of Chemiluminescent Ultra Sensitive AP Microwell and/or Membrane Substrate (450 nm), which had been 5-fold diluted with 1 mM MgCl₂-containing 20 mM TBS (pH 9.8) as a substrate, was added thereto. The resultant was incubated at room temperature under light-shielding for 40 minutes, and then luminescent intensity thereof was measured with an EnVisionTM multi-label counter. The EC₅₀ value of TIE-1-Ig γ 1-LALA with respect to the human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof were calculated. The relative value of luminescent intensity of 100 ng/mL TIE-1-Ig γ 1-LALA as maximum concentration point with respect to human Tie2 (1-452)-Fc chimeric protein and 23 mutant proteins thereof when the convergence value of the sigmoid curve of TIE-1-Ig γ 1-LALA binding to the human Tie2 (1-452)-Fc chimeric protein which was taken as 100% was calculated (Table 8 and Table 9). Further, the EC₅₀ value and the convergence value were calculated by Sigmoid-Emax model non-linear regression analysis. The results of ELISA are shown in Fig. 7.

[0173]

As a result, it has been found that compared with the human Tie2 (1-452)-Fc chimeric protein, TIE-1-Ig γ 1-LALA had a remarkably decreased relative value with respect to Tie2 (1-452)g1-Fc, Tie2 (1-452)g2-Fc and Tie2 (1-452)g4-Fc, which are mutant proteins. Further, it has been found that compared with the human Tie2 (1-452)-Fc chimeric protein, TIE-1-Ig γ 1-LALA had a decreased relative value and an increased EC₅₀ value with respect to Tie2 (1-452)g5-Fc, which is a mutant protein. From the result, it was found that TIE-1-Ig γ 1-LALA had a decreased binding activity to Tie2 (1-452)g1-Fc, Tie2 (1-452)g2-Fc, Tie2 (1-452)g4-Fc, and Tie2 (1-452)g5-Fc, unlike the human Tie2 (1-452)-Fc chimeric protein. Since TIE-1-Ig γ 1-LALA had a decreased relative value and similar EC₅₀ value with respect to Tie2 (1-452)A1-Fc, it was determined that TIE-1-Ig γ 1-LALA had no change in the binding activity to Tie2 (1-452)A1-Fc.

Table 8: Results of ELISA

[Table 8]

	Relative value (%) of luminescent intensity	EC ₅₀ value (ng/mL)
Human Tie2 (1-452)-Fc chimeric protein	97	1.1
Human Tie2 (1-452)r1-Fc	97	1.1
Human Tie2 (1-452)r2-Fc	96	0.9
Human Tie2 (1-452)r3-Fc	102	1.0
Human Tie2 (1-452)r4-Fc	101	1.0
Human Tie2 (1-452)r5-Fc	102	1.0
Human Tie2 (1-452)r6-Fc	100	1.0
Human Tie2 (1-452)r7-Fc	100	1.1
Human Tie2 (1-452)g1-Fc	3	12.3
Human Tie2 (1-452)g2-Fc	72	5.5
Human Tie2 (1-452)g3-Fc	96	1.1
Human Tie2 (1-452)g4-Fc	53	18
Human Tie2 (1-452)g5-Fc	91	2.2
Human Tie2 (1-452)g6-Fc	105	1.3
Human Tie2 (1-452)m1-Fc	104	1.0
Human Tie2 (1-452)m3-Fc	103	1.0
Human Tie2 (1-452)y1-Fc	109	1.0
Human Tie2 (1-452)c1-Fc	104	1.0

5

Table 9: Results of ELISA

[Table 9]

	Relative value (%) of luminescent intensity	EC ₅₀ value (ng/mL)
Human Tie2 (1-452)-Fc chimeric protein	98	1.0
Human Tie2 (1-452)A1-Fc	90	1.3
Human Tie2 (1-452)A2-Fc	95	1.3
Human Tie2 (1-452)A3-Fc	98	1.0
Human Tie2 (1-452)A4-Fc	100	1.0
Human Tie2 (1-452)A5-Fc	98	0.9
Human Tie2 (1-452)A6-Fc	99	1.1

[0174]

From the results of the two independent experiments, the ELISA and the SPR analysis, Tie2 (1-452)g1-Fc, Tie2 (1-452)g2-Fc, Tie2 (1-452)g4-Fc, and Tie2 (1-452)g5-Fc were identified as the mutant proteins to which the binding activity of TIE-1-Ig γ 1-LALA or the fully human 2-16A2-Fab was decreased in both experiments. It was found that the amino acids numbers 192, 194, 195, 197 and 198 in four mutant proteins are very important epitope candidates for TIE-1-Ig γ 1-LALA to bind to human Tie2. Herein, the binding activity of Tie2 (1-452)g1-Fc, which has the amino acid variations of I194A, N197A and L198A, decreased in ELISA assay, while the binding activity of Tie2 (1-452)g3-Fc which has the amino acid variation of I194A to TIE-1-Ig γ 1-LALA did not altered in ELISA assay. The binding activity of Tie2 (1-452)g6-Fc which has the amino acid variation of L198A altered neither in ELISA assay nor in SPR analysis. These results indicated that the mutation of amino acid number 197 in Tie2 (1-452)g1-Fc was the most critical amino acid as epitope. Finally, it was found that TIE-1-Ig γ 1-LALA binds to amino acid numbers 192, 195 and 197 of Accession No. NP_000450.2 as the epitopes.

Industrial Applicability

[0175]

The anti-human Tie2 antibody of the present invention is useful for preventing or treating various blood vessel-related diseases. Further, the polynucleotide, the expression vectors, the transformed host cell, and the methods for producing the antibody of the present invention are useful for producing the anti-human Tie2 antibody.

Sequence List Free Text

[0176]

In the number heading <223> of the sequence list below, description of "Artificial Sequence" is made. Specifically, the base sequence shown by SEQ ID NO: 1 in the sequence list is the base sequence of the heavy chain of TIE-1-Ig γ 1-LALA and the amino acid sequence shown by SEQ ID NO: 2 is the amino acid sequence of the heavy chain encoded by SEQ ID NO: 1. The base sequence shown by SEQ ID NO: 3 in the sequence list is the base sequence of the light chain of TIE-1-Ig γ 1-LALA, TIE-1-Ig γ 1-I253A, TIE-1-Ig γ 1-WT, TIE-1-Ig γ 4-PE, and fully human 2-16A2, and the amino acid sequence shown by SEQ ID NO: 4 is the amino acid sequence of the light chain encoded by SEQ ID NO: 3. The base sequence shown by SEQ ID NO: 5 in the sequence list is the base sequence of the heavy chain of TIE-1-Ig γ 1-I253A and the amino acid sequence shown by SEQ ID NO: 6 is the amino acid sequence of the heavy chain encoded by SEQ ID NO: 5. The base sequence shown by SEQ ID NO: 7 in the sequence list is the base sequence of the heavy chain of TIE-1-Ig γ 1-WT, and the amino acid sequence shown by SEQ ID NO: 8 is the

amino acid sequence of the heavy chain encoded by SEQ ID NO: 7. The base sequence shown by SEQ ID NO: 9 in the sequence list is the base sequence of the heavy chain of TIE-1-Ig γ 4-PE, and the amino acid sequence shown by SEQ ID NO: 10 is the amino acid sequence of the heavy chain encoded by SEQ ID NO: 9. The base sequence shown by

5 SEQ ID NO: 11 in the sequence list is the base sequence of the heavy chain of the fully human 2-16A2, and the amino acid sequence shown by SEQ ID NO: 12 is the amino acid sequence of the heavy chain encoded by SEQ ID NO: 11. The amino acid sequences shown by SEQ ID NOS: 13 to 20 in the sequence list are the amino acid sequences of the linker. The amino acid sequence shown by SEQ ID NO: 22 in the sequence list is a

10 thrombin recognition site.

Claims

[Claim 1] An anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, wherein

the heavy chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of the amino acid numbers 99 to 111 of SEQ ID NO: 2;

the light chain variable region comprises CDR1 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of the amino acid numbers 94 to 102 of SEQ ID NO: 4; and

the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment comprises four antigen-binding sites.

[Claim 2] The anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 1, selected from (1) or (2) below:

(1) an anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising four heavy chain variable regions and four light chain variable regions, in which

the heavy chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2,

the light chain variable region consists of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and

the one heavy chain variable region and the one light chain variable region constitute one antigen-binding site, and the antibody or the antigen-binding fragment comprises four antigen-binding sites; and

(2) an anti-human Tie2 antibody or an antigen-binding fragment thereof which is an antibody or an antigen-binding fragment thereof derived from posttranslational modification of the anti-human Tie2 antibody or the antigen-binding fragment thereof of (1).

[Claim 3] The anti-human Tie2 antibody according to claim 1, wherein the antibody comprises two heavy chains and four light chains;

each heavy chain comprises two structures consisting of a heavy chain variable region comprising CDR1 consisting of the amino acid sequence of the amino acid numbers 31 to 35 of SEQ ID NO: 2, CDR2 consisting of the amino acid sequence of the amino acid numbers 50 to 66 of SEQ ID NO: 2, and CDR3 consisting of the amino acid sequence of the amino acid numbers 99 to 111 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the carboxy terminus (C terminus) of one of the structures is linked to the amino terminus (N terminus) of the other structure through a linker; and

each light chain comprises a light chain variable region comprising CDR1 consisting of the amino acid sequence of the amino acid numbers 24 to 39 of SEQ ID NO: 4, CDR2 consisting of the amino acid sequence of the amino acid numbers 55 to 61 of SEQ ID NO: 4, and CDR3 consisting of the amino acid sequence of the amino acid numbers 94 to 102 of SEQ ID NO: 4, and a light chain constant region.

[Claim 4] The anti-human Tie2 antibody according to claim 3, selected from (1) or (2) below:

(1) an anti-human Tie2 antibody comprising two heavy chains and four light chains, in which

each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker; and

each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region; and

(2) an anti-human Tie2 antibody, which is an antibody derived from posttranslational modification of the anti-human Tie2 antibody of (1).

[Claim 5] The anti-human Tie2 antibody according to claim 4, wherein

the anti-human Tie2 antibody comprises two heavy chains and four light chains;

each heavy chain comprises two structures consisting of a heavy chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 122 of SEQ ID NO: 2 and a CH1 region, a CH2 region, and a CH3 region, and the C terminus of one of the structures is linked to the N terminus of the other structure through a linker; and

each light chain comprises a light chain variable region consisting of the amino acid sequence of the amino acid numbers 1 to 113 of SEQ ID NO: 4, and a light chain constant region.

[Claim 6] An anti-human Tie2 antibody which is an antibody derived from

posttranslational modification of the anti-human Tie2 antibody according to claim 5.

[Claim 7] The anti-human Tie2 antibody according to claim 6, wherein the posttranslational modification is pyroglutamylation at the N terminus of the heavy chain variable region and/or deletion of lysine at the C terminus of the heavy chain.

[Claim 8] The anti-human Tie2 antibody according to claim 4, comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[Claim 9] The anti-human Tie2 antibody according to claim 4, comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 6 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[Claim 10] The anti-human Tie2 antibody according to claim 4, comprising two heavy chains consisting of the amino acid sequence shown by SEQ ID NO: 10 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[Claim 11] An anti-human Tie2 antibody which is an antibody derived from posttranslational modification of the anti-human Tie2 antibody according to any one of claims 8 to 10.

[Claim 12] The anti-human Tie2 antibody according to claim 11, wherein the posttranslational modification is pyroglutamylation at the N terminus of the heavy chain variable region and/or deletion of lysine at the C terminus of the heavy chain.

[Claim 13] The anti-human Tie2 antibody according to claim 11, comprising two heavy chains consisting of the amino acid sequence of the amino acid numbers 1 to 678 of SEQ ID NO: 2 and four light chains consisting of the amino acid sequence shown by SEQ ID NO: 4.

[Claim 14] A tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof, binding to the same human Tie2 epitope as the anti-human Tie2 antibody according to claim 8 or 13.

[Claim 15] The tetravalent anti-human Tie2 antibody or the antigen-binding fragment thereof according to Claim 14, wherein the human Tie2 epitope is the human Tie2 epitope containing the amino acid of the amino acid numbers 192, 195 and 197 of Accession No.

NP_000450.2.

[Claim 16] A polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof
5 according to claim 2.

[Claim 17] A polynucleotide comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof
10 according to claim 2.

[Claim 18] A polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10.

[Claim 19] A polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody according to any one of claims 8 to 10.
15

[Claim 20] An expression vector comprising the polynucleotide according to claim 16 and/or 17.

20 [Claim 21] An expression vector comprising the polynucleotide according to claim 18 and/or 19.

[Claim 22] A host cell transformed with the expression vector according to claim 20, which is selected from the group consisting of (a) to (d) below:

25 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2, and a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or an antigen-binding fragment thereof;

30 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or an antigen-binding fragment thereof;

35 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2; and

(d) a host cell transformed with an expression vector comprising a polynucleotide

comprising a base sequence encoding the light chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2.

[Claim 23] A host cell transformed with the expression vector according to claim 21,
5 selected from the group consisting of (a) to (d) below:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10 and a polynucleotide comprising a base sequence encoding the light chain of the antibody;

10 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody;

(c) a host cell transformed with an expression vector comprising a polynucleotide
15 comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10; and

(d) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody according to any one of claims 8 to 10.

20

[Claim 24] A method for producing an anti-human Tie2 antibody or an antigen-binding fragment thereof, comprising culturing host cell(s) selected from the group consisting of (a) to (c) below to express a tetravalent anti-human Tie2 antibody or an antigen-binding fragment thereof:

25 (a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2 and a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof;

30 (b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof; and

35 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain variable region of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to claim 2 and a host cell transformed with an expression vector comprising a polynucleotide comprising a base

sequence encoding the light chain variable region of the antibody or the antigen-binding fragment thereof.

[Claim 25] A method for producing an anti-human Tie2 antibody, comprising
5 culturing host cell(s) selected from the group consisting of (a) to (c) below to express an anti-human Tie2 antibody:

(a) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10 and a polynucleotide comprising a base sequence
10 encoding the light chain of the antibody;

(b) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10 and an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the antibody; and

15 (c) a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the heavy chain of the anti-human Tie2 antibody according to any one of claims 8 to 10 and a host cell transformed with an expression vector comprising a polynucleotide comprising a base sequence encoding the light chain of the anti-human Tie2 antibody.

20 [Claim 26] An anti-human Tie2 antibody or an antigen-binding fragment thereof, produced by the method according to claim 24.

[Claim 27] An anti-human Tie2 antibody produced by the method according to claim
25 25.

[Claim 28] A pharmaceutical composition comprising the anti-human Tie2 antibody or the antigen-binding fragment thereof according to any one of claims 1 to 13, 26, and 27, and a pharmaceutically acceptable excipient.

30 [Claim 29] A pharmaceutical composition comprising the anti-human Tie2 antibody according to claim 5, the anti-human Tie2 antibody according to claim 6, and a pharmaceutically acceptable excipient.

35 [Claim 30] A pharmaceutical composition comprising the anti-human Tie2 antibody according to claim 8, the anti-human Tie2 antibody according to claim 13, and a pharmaceutically acceptable excipient.

[Claim 31] The pharmaceutical composition according to any one of claims 28 to 30, which is a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

5 [Claim 32] A method for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia, comprising administering a therapeutically effective amount of the anti-human Tie2 antibody or the antigen-binding fragment thereof according to any one of claims 1 to 13, 26, and 27.

10 [Claim 33] The anti-human Tie2 antibody or the antigen-binding fragment thereof according to any one of claims 1 to 13, 26, and 27, for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

[Claim 34] Use of the anti-human Tie2 antibody or the antigen-binding fragment
15 thereof according to any one of claims 1 to 13, 26, and 27 for manufacture of a pharmaceutical composition for preventing or treating diabetic macular edema, diabetic retinopathy, or critical limb ischemia.

SEQUENCE LISTING

<110> Astellas Pharma Inc.
 <120> Novel anti-human Tie2 antibody
 <130> A15017A00
 <150> JP2014-145135
 <151> 2014-07-15
 <160> 23
 <170> PatentIn version 3.5
 <210> 1
 <211> 2037
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> H-chain of anti-human Tie2 antibody

<220>
 <221> CDS
 <222> (1).. (2037)

<400> 1
 gaa gtg cag ctg gtg gaa tct ggc ggc gga ctg gtg cag cct ggc gga 48
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 tct ctg aga ctg tct tgt gcc gcc tcc ggc ttc acc ttc gac gac tac 96
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 gct atg cac tgg gtg cga cag gcc cct ggc aag gga ctg gaa tgg gtg 144
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 gcc ggc atc tcc tgg aac tcc ggc tct atc gtg tac gcc gac tcc gtg 192
 Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val
 50 55 60
 aag ggc cgg ttc acc atc tcc cgg gac aac tcc aag aac acc ctg tac 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 ctg cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc 288
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 gct aag gac atc cgg gaa cag ctg gtg gaa gat gcc ttc gac atc tgg 336
 Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
 100 105 110

ggc Gly	cag Gln	ggc Gly 115	acc Thr	ctc Leu	gtg Val	acc Thr 120	gtg Val	tcc Ser	tot Ser	gct Ala	tot Ser 125	acc Thr	aag Lys	ggc Gly	ccc Pro	384
tcc Ser 130	gtg Val	ttc Phe	cct Pro	ctg Leu	gcc Ala 135	cct Pro	tcc Ser	agc Ser	aag Lys	tot Ser 140	acc Thr	tot Ser	ggc Gly	ggc Gly	aca Thr	432
gcc Ala 145	gct Ala	ctg Leu	ggc Gly	tgc Cys 150	ctc Leu	gtg Val	aag Lys	gac Asp	tac Tyr	ttc Phe 155	ccc Pro	gag Glu	ccc Pro	gtg Val	aca Thr 160	480
gtg Val	tct Ser	tgg Trp	aac Asn 165	tct Ser	ggc Gly	gcc Ala	ctg Leu	aca Thr 170	tct Ser	ggc Gly	gtg Val	cac His	acc Thr	ttc Phe 175	cct Pro	528
gct Ala	gtg Val	ctg Leu	cag Gln 180	tcc Ser	tcc Ser	ggc Gly	ctg Leu	tac Tyr 185	tcc Ser	ctg Leu	tcc Ser	tcc Ser	gtc Val 190	gtg Val	act Thr	576
gtg Val	ccc Pro 195	tcc Ser	agc Ser	tct Ser	ctg Leu	ggc Gly	acc Thr 200	cag Gln	acc Thr	tac Tyr	atc Ile 205	tgc Cys	aac Asn	gtg Val	aac Asn	624
cac His 210	aag Lys	ccc Pro	tcc Ser	aac Asn	acc Thr	aag Lys 215	gtg Val	gac Asp	aag Lys	aag Lys	gtg Val 220	gaa Glu	ccc Pro	aag Lys	tcc Ser	672
tgc Cys 225	ggc Gly	agc Ser	gag Glu	gtg Val	cag Gln 230	ctg Leu	gtg Val	gaa Glu	agt Ser	ggg Gly 235	gga Gly	ggc Gly	ctg Leu	gtg Val	cag Gln 240	720
cca Pro	ggt Gly	gga Gly	agc Ser	ctg Leu 245	aga Arg	ctg Leu	agc Ser	tgc Cys	gcc Ala 250	gct Ala	tot Ser	ggc Gly	ttt Phe 255	acc Thr 255	ttt Phe	768
gat Asp	gat Asp	tat Tyr	gcc Ala 260	atg Met	cat His	tgg Trp	gtg Val	cgc Arg 265	cag Gln	gct Ala	cca Pro	ggg Gly	aaa Lys 270	ggc Gly	ctg Leu	816
gaa Glu	tgg Trp	gtg Val 275	gca Ala	ggg Gly	atc Ile	agc Ser	tgg Trp 280	aac Asn	agc Ser	ggc Gly	agc Ser	atc Ile 285	gtg Val	tat Tyr	gct Ala	864
gat Asp 290	agc Ser	gtg Val	aag Lys	ggg Gly	cgc Arg	ttt Phe 295	aca Thr	atc Ile	agc Ser	aga Arg	gac Asp 300	aac Asn	agc Ser	aag Lys	aat Asn	912
act Thr 305	ctg Leu	tac Tyr	ctg Leu	cag Gln	atg Met 310	aat Asn	agc Ser	ctg Leu	cgc Arg	gct Ala 315	gag Glu	gat Asp	aca Thr	gct Ala	gtg Val 320	960
tat Tyr	tac Tyr	tgt Cys	gcc Ala 325	aag Lys	gat Asp	att Ile	cgc Arg	gag Glu	cag Gln 330	ctg Leu	gtg Val	gaa Glu	gat Asp	gct Ala 335	ttt Phe	1008
gat Tyr	att Tyr	tgg Cys	ggg Gly	cag Gln	ggt Asp	aca Thr	ctc Leu	gtg Val	aca Thr	gtg Val	tct Ser	agc Ser	gcc Ala	tcc Ser	acc Thr	1056

Asp	Ile	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr		
			340					345					350				
aag	ggc	cca	agc	gtg	ttc	cca	ctg	gct	ccc	agc	tcc	aag	agc	acc	tcc		1104
Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser		
		355					360					365					
ggt	gga	act	gct	gcc	ctg	ggc	tgt	ctc	gtg	aaa	gat	tat	ttt	cct	gag		1152
Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu		
	370					375					380						
cct	gtg	act	gtg	tcc	tgg	aat	agc	ggc	gct	ctg	acc	agc	gga	gtg	cat		1200
Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His		
385					390					395					400		
acc	ttt	cca	gcc	gtg	ctg	cag	agc	agc	ggc	ctg	tat	agc	ctg	tcc	agc		1248
Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser		
			405						410					415			
gtc	gtg	acc	gtg	cct	tcc	tct	agc	ctg	gga	aca	cag	aca	tat	atc	tgt		1296
Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys		
			420					425					430				
aat	gtg	aat	cat	aag	ccc	agt	aat	acc	aaa	gtg	gat	aag	aaa	gtg	gaa		1344
Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu		
		435					440					445					
cct	aag	agc	tgc	gac	aag	acc	cac	acc	tgt	ccc	cct	tgt	cct	gcc	cct		1392
Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro		
	450					455					460						
gaa	gct	gct	ggc	ggc	cct	tct	gtg	ttt	ctg	ttc	ccc	cca	aag	cct	aag		1440
Glu	Ala	Ala	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys		
465					470				475						480		
gac	acc	ctg	atg	atc	tcc	cgg	acc	ccc	gaa	gtg	acc	tgc	gtg	gtg	gtg		1488
Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val		
			485						490				495				
gat	gtg	tcc	cac	gag	gac	cct	gaa	gtg	aag	ttc	aat	tgg	tac	gtg	gac		1536
Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp		
			500					505					510				
ggc	gtg	gaa	gtg	cac	aac	gcc	aag	acc	aag	cct	aga	gag	gaa	cag	tac		1584
Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr		
		515					520					525					
aac	tcc	acc	tac	cgg	gtg	gtg	tcc	gtg	ctg	aca	gtg	ctg	cat	cag	gac		1632
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp		
	530					535					540						
tgg	ctg	aac	ggc	aaa	gag	tac	aag	tgc	aag	gtg	tcc	aac	aag	gcc	ctg		1680
Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu		
545					550					555					560		
ccc	gcc	tcc	atc	gaa	aag	acc	atc	tcc	aag	gcc	aag	ggc	cag	ccc	cgg		1728
Pro	Ala	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg		

565					570					575						
gaa	ccc	cag	gtg	tac	aca	ctg	ccc	cct	agc	agg	gac	gag	ctg	acc	aag	1776
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	
			580					585					590			
aac	cag	gtg	tcc	ctg	acc	tgt	ctc	gtg	aag	ggc	ttc	tac	ccc	tcc	gat	1824
Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
		595					600					605				
atc	gcc	gtg	gaa	tgg	gag	tcc	aac	ggc	cag	cct	gag	aac	aac	tat	aag	1872
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	
	610					615					620					
acc	acc	ccc	cct	gtg	ctg	gac	tcc	gac	ggc	tca	ttc	ttt	ctg	tac	tcc	1920
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	
	625				630				635						640	
aag	ctg	acc	gtg	gac	aag	tcc	cgg	tgg	cag	cag	ggc	aac	gtg	ttc	tcc	1968
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	
			645					650					655			
tgc	agc	gtg	atg	cac	gag	gcc	ctg	cac	aac	cac	tac	acc	cag	aag	tcc	2016
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	
		660					665						670			
ctg	tcc	ctg	agc	ccc	ggc	aaa										2037
Leu	Ser	Leu	Ser	Pro	Gly	Lys										
		675														

<210> 2
 <211> 679
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 2

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
1				5					10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	Asp	Tyr	
			20					25					30			
Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
		35					40					45				
Ala	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Ser	Ile	Val	Tyr	Ala	Asp	Ser	Val	
	50					55					60					

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
225 230 235 240

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
245 250 255

Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
260 265 270

Glu Trp Val Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala
275 280 285

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn

290

295

300

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
 305 310 315 320

Tyr Tyr Cys Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe
 325 330 335

Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
 340 345 350

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 355 360 365

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 370 375 380

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 385 390 395 400

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 405 410 415

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 420 425 430

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
 435 440 445

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 450 455 460

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 465 470 475 480

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 485 490 495

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 500 505 510

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 515 520 525

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
530 535 540

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
545 550 555 560

Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
565 570 575

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
580 585 590

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
595 600 605

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
610 615 620

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
625 630 635 640

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
645 650 655

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
660 665 670

Leu Ser Leu Ser Pro Gly Lys
675

<210> 3
<211> 657
<212> DNA
<213> Artificial Sequence

<220>
<223> L-chain of anti-human Tie2 antibody

<220>
<221> CDS
<222> (1).. (657)

<400> 3
gac ata caa atg acc cag tct cca tcc tca ctt agt gcc tct gtt ggc

Asp 1	Ile	Gln	Met	Thr 5	Gln	Ser	Pro	Ser	Ser 10	Leu	Ser	Ala	Ser	Val 15	Gly	
gat	aga	gta	act	atc	aca	tgc	cgg	tcc	tcc	caa	tct	ctg	ctg	cat	tcc	96
Asp	Arg	Val	Thr 20	Ile	Thr	Cys	Arg	Ser 25	Ser	Gln	Ser	Leu	Leu	His	Ser	
cac	cgc	tac	aac	tac	ctg	gac	tgg	tat	cag	cag	aaa	ccc	ggc	aaa	gct	144
His	Arg	Tyr 35	Asn	Tyr	Leu	Asp	Trp 40	Tyr	Gln	Gln	Lys	Pro 45	Gly	Lys	Ala	
ccc	aag	ctc	ttg	att	tac	ctg	ggg	tct	aat	agg	gca	agc	ggt	gtc	cca	192
Pro	Lys 50	Leu	Leu	Ile	Tyr	Leu 55	Gly	Ser	Asn	Arg	Ala 60	Ser	Gly	Val	Pro	
agt	cga	ttc	agc	gga	agc	ggg	agc	ggc	aca	gat	ttt	aca	ctc	act	atc	240
Ser 65	Arg	Phe	Ser	Gly	Ser 70	Gly	Ser	Gly	Thr	Asp 75	Phe	Thr	Leu	Thr	Ile 80	
agt	tca	gtg	cag	cct	gag	gac	ttc	gcc	acc	tat	tat	tgt	atg	cag	act	288
Ser	Ser	Val	Gln	Pro 85	Glu	Asp	Phe	Ala	Thr 90	Tyr	Tyr	Cys	Met	Gln	Thr 95	
ctg	cag	acc	cct	ctg	acc	ttt	ggt	cag	gga	acc	aag	gtg	gaa	atc	aag	336
Leu	Gln	Thr	Pro 100	Leu	Thr	Phe	Gly	Gln 105	Gly	Thr	Lys	Val	Glu 110	Ile	Lys	
cgt	acg	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	cgc	cca	tct	gat	gag	384
Arg	Thr	Val 115	Ala	Ala	Pro	Ser	Val 120	Phe	Ile	Phe	Pro	Pro 125	Ser	Asp	Glu	
cag	ttg	aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	432
Gln	Leu 130	Lys	Ser	Gly	Thr	Ala 135	Ser	Val	Val	Cys	Leu 140	Leu	Asn	Asn	Phe	
tat	ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	480
Tyr 145	Pro	Arg	Glu	Ala	Lys 150	Val	Gln	Trp	Lys	Val 155	Asp	Asn	Ala	Leu	Gln 160	
tcg	ggt	aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	528
Ser	Gly	Asn	Ser	Gln 165	Glu	Ser	Val	Thr	Glu 170	Gln	Asp	Ser	Lys	Asp 175	Ser	
acc	tac	agc	ctg	agc	agc	acc	ctg	acg	ctg	agc	aaa	gca	gac	tac	gag	576
Thr	Tyr	Ser	Leu 180	Ser	Ser	Thr	Leu	Thr 185	Leu	Ser	Lys	Ala	Asp 190	Tyr	Glu	
aaa	cac	aaa	gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	ggc	ctg	agc	tgc	624
Lys	His	Lys 195	Val	Tyr	Ala	Cys	Glu 200	Val	Thr	His	Gln	Gly 205	Leu	Ser	Ser	
ccc	gtc	aca	aag	agc	ttc	aac	agg	gga	gag	tgt						657
Pro	Val 210	Thr	Lys	Ser	Phe	Asn 215	Arg	Gly	Glu	Cys						

<210> 4

<211> 219
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 4

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

His Arg Tyr Asn Tyr Leu Asp Trp Tyr Gln Gln Lys Pro Gly Lys Ala
 35 40 45

Pro Lys Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 65 70 75 80

Ser Ser Val Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Met Gln Thr
 85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 5
<211> 2037
<212> DNA
<213> Artificial Sequence

<220>
<223> H-chain of anti-human Tie2 antibody

<220>
<221> CDS
<222> (1).. (2037)

<400> 5
gaa gtg cag ctg gtg gaa tct ggc ggc gga ctg gtg cag cct ggc gga 48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
tct ctg aga ctg tct tgt gcc gcc tcc ggc ttc acc ttc gac gac tac 96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30
gct atg cac tgg gtg cga cag gcc cct ggc aag gga ctg gaa tgg gtg 144
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
gcc ggc atc tcc tgg aac tcc ggc tct atc gtg tac gcc gac tcc gtg 192
Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val
50 55 60
aag ggc cgg ttc acc atc tcc cgg gac aac tcc aag aac acc ctg tac 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
ctg cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc 288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
gct aag gac atc cgg gaa cag ctg gtg gaa gat gcc ttc gac atc tgg 336
Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110
ggc cag ggc acc ctc gtg acc gtg tcc tct gct tct acc aag ggc ccc 384
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125
tcc gtg ttc cct ctg gcc cct tcc agc aag tct acc tct ggc ggc aca 432
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

gcc gct ctg ggc tgc ctc gtg aag gac tac ttc ccc gag ccc gtg aca Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr 145 150 155 160	480
gtg tct tgg aac tct ggc gcc ctg aca tct ggc gtg cac acc ttc cct Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro 165 170 175	528
gct gtg ctg cag tcc tcc ggc ctg tac tcc ctg tcc tcc gtc gtg act Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr 180 185 190	576
gtg ccc tcc agc tct ctg ggc acc cag acc tac atc tgc aac gtg aac Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn 195 200 205	624
cac aag ccc tcc aac acc aag gtg gac aag aag gtg gaa ccc aag tcc His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser 210 215 220	672
tgc ggc agc gag gtg cag ctg gtg gaa agt ggg gga ggc ctg gtg cag Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln 225 230 235 240	720
cca ggt gga agc ctg aga ctg agc tgc gcc gct tot ggc ttt acc ttt Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe 245 250 255	768
gat gat tat gcc atg cat tgg gtg cgc cag gct cca ggg aaa ggc ctg Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu 260 265 270	816
gaa tgg gtg gca ggg atc agc tgg aac agc ggc agc atc gtg tat gct Glu Trp Val Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala 275 280 285	864
gat agc gtg aag ggg cgc ttt aca atc agc aga gac aac agc aag aat Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn 290 295 300	912
act ctg tac ctg cag atg aat agc ctg cgc gct gag gat aca gct gtg Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val 305 310 315 320	960
tat tac tgt gcc aag gat att cgc gag cag ctg gtg gaa gat gct ttt Tyr Tyr Cys Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe 325 330 335	1008
gat att tgg ggg cag ggt aca ctc gtg aca gtg tct agc gcc tcc acc Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr 340 345 350	1056
aag ggc cca agc gtg ttc cca ctg gct ccc agc tcc aag agc acc tcc Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 355 360 365	1104

ggt gga act gct gcc ctg ggc tgt ctc gtg aaa gat tat ttt cct gag Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 370 375 380	1152
cct gtg act gtg tcc tgg aat agc ggc gct ctg acc agc gga gtg cat Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 385 390 395 400	1200
acc ttt cca gcc gtg ctg cag agc agc ggc ctg tat agc ctg tcc agc Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 405 410 415	1248
gtc gtg acc gtg cct tcc tct agc ctg gga aca cag aca tat atc tgt Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys 420 425 430	1296
aat gtg aat cat aag ccc agt aat acc aaa gtg gat aag aaa gtg gaa Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 435 440 445	1344
cct aag agc tgc gac aag acc cac acc tgt ccc cct tgt cct gcc cct Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro 450 455 460	1392
gaa gct gct ggc ggc cct tct gtg ttt ctg ttc ccc cca aag cct aag Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys 465 470 475 480	1440
gac acc ctg atg gcc tcc cgg acc ccc gaa gtg acc tgc gtg gtg gtg Asp Thr Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 485 490 495	1488
gat gtg tcc cac gag gac cct gaa gtg aag ttc aat tgg tac gtg gac Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp 500 505 510	1536
ggc gtg gaa gtg cac aac gcc aag acc aag cct aga gag gaa cag tac Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 515 520 525	1584
aac tcc acc tac cgg gtg gtg tcc gtg ctg aca gtg ctg cat cag gac Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp 530 535 540	1632
tgg ctg aac ggc aaa gag tac aag tgc aag gtg tcc aac aag gcc ctg Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu 545 550 555 560	1680
ccc gcc tcc atc gaa aag acc atc tcc aag gcc aag ggc cag ccc cgg Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg 565 570 575	1728
gaa ccc cag gtg tac aca ctg ccc cct agc agg gac gag ctg acc aag Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys 580 585 590	1776
aac cag gtg tcc ctg acc tgt ctc gtg aag ggc ttc tac ccc tcc gat	1824

Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp		
		595					600					605					
atc	gcc	gtg	gaa	tgg	gag	tcc	aac	ggc	cag	cct	gag	aac	aac	tat	aag		1872
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys		
	610					615					620						
acc	acc	ccc	cct	gtg	ctg	gac	tcc	gac	ggc	tca	ttc	ttt	ctg	tac	tcc		1920
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser		
625					630					635					640		
aag	ctg	acc	gtg	gac	aag	tcc	cgg	tgg	cag	cag	ggc	aac	gtg	ttc	tcc		1968
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser		
				645					650					655			
tgc	agc	gtg	atg	cac	gag	gcc	ctg	cac	aac	cac	tac	acc	cag	aag	tcc		2016
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser		
			660					665					670				
ctg	tcc	ctg	agc	ccc	ggc	aaa											2037
Leu	Ser	Leu	Ser	Pro	Gly	Lys											
		675															

<210> 6
 <211> 679
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 6

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly		
1				5					10					15			
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	Asp	Tyr		
			20					25					30				
Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val		
		35					40					45					
Ala	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Ser	Ile	Val	Tyr	Ala	Asp	Ser	Val		
	50					55					60						
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr		
65					70					75					80		
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys		
				85					90					95			

Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
225 230 235 240

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
245 250 255

Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
260 265 270

Glu Trp Val Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala
275 280 285

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
290 295 300

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
305 310 315 320

Tyr Tyr Cys Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe
325 330 335

Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
340 345 350

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
355 360 365

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
370 375 380

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
385 390 395 400

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
405 410 415

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
420 425 430

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
435 440 445

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
450 455 460

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
465 470 475 480

Asp Thr Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
485 490 495

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
500 505 510

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
515 520 525

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
530 535 540

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu

gcc atg cac tgg gtc cga cag gcc cct ggc aag ggc ctg gaa tgg gtg Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45	144
gcc ggc atc tcc tgg aac tcc ggc tcc atc gtg tac gcc gac tcc gtg Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val 50 55 60	192
aag ggc cgg ttc acc atc tcc cgg gac aac tcc aag aac acc ctg tac Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80	240
ctg cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95	288
gcc aag gac atc aga gag cag ctg gtc gag gac gcc ttc gac atc tgg Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp 100 105 110	336
ggc cag ggc acc ctg gtc acc gtg tcc tca gcc tcc acc aag ggc cca Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro 115 120 125	384
tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr 130 135 140	432
gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr 145 150 155 160	480
gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro 165 170 175	528
gct gtc cta cag tcc tca gga ctc tac tcc ctt agt agc gtg gtg acc Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr 180 185 190	576
gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn 195 200 205	624
cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc aaa tct His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser 210 215 220	672
tgt gga tcc gag gtg cag ctg gtg gaa tcc ggc gga ggc ctg gtg cag Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln 225 230 235 240	720
cct ggc ggc tct ctg aga ctg tcc tgt gcc gcc tcc ggc ttc acc ttc Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe 245 250 255	768
gac gac tac gcc atg cac tgg gtc cga cag gcc cct ggc aag ggc ctg	816

Asp	Asp	Tyr	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
			260					265					270			
gaa	tgg	gtg	gcc	ggc	atc	tcc	tgg	aac	tcc	ggc	tcc	atc	gtg	tac	gcc	864
Glu	Trp	Val	Ala	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Ser	Ile	Val	Tyr	Ala	
		275					280					285				
gac	tcc	gtg	aag	ggc	cgg	ttc	acc	atc	tcc	cgg	gac	aac	tcc	aag	aac	912
Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	
	290					295					300					
acc	ctg	tac	ctg	cag	atg	aac	tcc	ctg	cgg	gcc	gag	gac	acc	gcc	gtg	960
Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	
305					310					315					320	
tac	tac	tgc	gcc	aag	gac	atc	aga	gag	cag	ctg	gtc	gag	gac	gcc	ttc	1008
Tyr	Tyr	Cys	Ala	Lys	Asp	Ile	Arg	Glu	Gln	Leu	Val	Glu	Asp	Ala	Phe	
				325					330					335		
gac	atc	tgg	ggc	cag	ggc	acc	ctg	gtc	acc	gtg	tcc	tca	gcc	tcc	acc	1056
Asp	Ile	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	
			340					345					350			
aag	ggc	cca	tgc	gtc	ttc	ccc	ctg	gca	ccc	tcc	tcc	aag	agc	acc	tct	1104
Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	
		355					360					365				
ggg	ggc	aca	gcg	gcc	ctg	ggc	tgc	ctg	gtc	aag	gac	tac	ttc	ccc	gaa	1152
Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	
		370				375					380					
ccg	gtg	acg	gtg	tgc	tgg	aac	tca	ggc	gcc	ctg	acc	agc	ggc	gtg	cac	1200
Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	
385					390					395					400	
acc	ttc	ccg	gct	gtc	cta	cag	tcc	tca	gga	ctc	tac	tcc	ctt	agt	agc	1248
Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	
				405					410					415		
gtg	gtg	acc	gtg	ccc	tcc	agc	agc	ttg	ggc	acc	cag	acc	tac	atc	tgc	1296
Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	
			420					425					430			
aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	gac	aag	aaa	gtt	gag	1344
Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	
		435					440					445				
ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	gca	cct	1392
Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	
		450				455					460					
gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	1440
Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	
465					470				475						480	
gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	1488
Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	

485					490					495						
gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	1536
Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	
			500					505					510			
ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	1584
Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	
		515					520					525				
aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	1632
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	
	530					535					540					
tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	1680
Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	
545					550					555					560	
cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggg	cag	ccc	cga	1728
Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	
				565					570					575		
gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	1776
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	
			580					585					590			
aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	1824
Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
		595					600					605				
atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	tac	aag	1872
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	
	610					615					620					
acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	1920
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	
625					630					635					640	
aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	1968
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	
			645						650				655			
tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	tac	acg	cag	aag	agc	2016
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	
			660					665					670			
ctc	tcc	ctg	tct	ccg	ggt	aaa										2037
Leu	Ser	Leu	Ser	Pro	Gly	Lys										
		675														

<210> 8
 <211> 679
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 8

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
20

210

215

220

Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
 225 230 235 240

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 245 250 255

Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 260 265 270

Glu Trp Val Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala
 275 280 285

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
 290 295 300

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
 305 310 315 320

Tyr Tyr Cys Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe
 325 330 335

Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
 340 345 350

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 355 360 365

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 370 375 380

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 385 390 395 400

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 405 410 415

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 420 425 430

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
 435 440 445

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
450 455 460

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
465 470 475 480

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
485 490 495

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
500 505 510

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
515 520 525

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
530 535 540

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
545 550 555 560

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
565 570 575

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
580 585 590

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
595 600 605

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
610 615 620

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
625 630 635 640

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
645 650 655

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
660 665 670

Leu Ser Leu Ser Pro Gly Lys
675

<210> 9
<211> 2028
<212> DNA
<213> Artificial Sequence

<220>
<223> H-chain of anti-human Tie2 antibody

<220>
<221> CDS
<222> (1).. (2028)

<400> 9	
gag gtg cag ctg gtg gaa tcc ggc gga ggc ctg gtg cag cct ggc ggc	48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
tct ctg aga ctg tcc tgt gcc gcc tcc ggc ttc acc ttc gac gac tac	96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr	
20 25 30	
gcc atg cac tgg gtc cga cag gcc cct ggc aag ggc ctg gaa tgg gtg	144
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
gcc ggc atc tcc tgg aac tcc ggc tcc atc gtg tac gcc gac tcc gtg	192
Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val	
50 55 60	
aag ggc cgg ttc acc atc tcc cgg gac aac tcc aag aac acc ctg tac	240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	
65 70 75 80	
ctg cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc	288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
gcc aag gac atc aga gag cag ctg gtc gag gac gcc ttc gac atc tgg	336
Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp	
100 105 110	
ggc cag ggc acc ctg gtc acc gtg tcc tca gcc tcc acc aag ggc cca	384
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
115 120 125	
tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca	432
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	
130 135 140	
gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg	480
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr	

145					150					155					160	
gtg	tcg	tgg	aac	tca	ggc	gcc	ctg	acc	agc	ggc	gtg	cac	acc	ttc	cgc	528
Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	
				165					170					175		
gct	gtc	cta	cag	tcc	tca	gga	ctc	tac	tcc	ctt	agt	agc	gtg	gtg	acc	576
Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	
			180					185					190			
gtg	ccc	tcc	agc	agc	ttg	ggc	acc	cag	acc	tac	atc	tgc	aac	gtg	aat	624
Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	
		195					200					205				
cac	aag	ccc	agc	aac	acc	aag	gtg	gac	aag	aaa	gtt	gag	ccc	aaa	tct	672
His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	
	210					215					220					
tgt	gga	tcc	gag	gtg	cag	ctg	gtg	gaa	tcc	ggc	gga	ggc	ctg	gtg	cag	720
Cys	Gly	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	
225					230					235					240	
cct	ggc	ggc	tct	ctg	aga	ctg	tcc	tgt	gcc	gcc	tcc	ggc	ttc	acc	ttc	768
Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	
				245					250					255		
gac	gac	tac	gcc	atg	cac	tgg	gtc	cga	cag	gcc	cct	ggc	aag	ggc	ctg	816
Asp	Asp	Tyr	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
			260					265					270			
gaa	tgg	gtg	gcc	ggc	atc	tcc	tgg	aac	tcc	ggc	tcc	atc	gtg	tac	gcc	864
Glu	Trp	Val	Ala	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Ser	Ile	Val	Tyr	Ala	
		275					280					285				
gac	tcc	gtg	aag	ggc	cgg	ttc	acc	atc	tcc	cgg	gac	aac	tcc	aag	aac	912
Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	
	290					295					300					
acc	ctg	tac	ctg	cag	atg	aac	tcc	ctg	cgg	gcc	gag	gac	acc	gcc	gtg	960
Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	
305				310						315					320	
tac	tac	tgc	gcc	aag	gac	atc	aga	gag	cag	ctg	gtc	gag	gac	gcc	ttc	1008
Tyr	Tyr	Cys	Ala	Lys	Asp	Ile	Arg	Glu	Gln	Leu	Val	Glu	Asp	Ala	Phe	
				325					330					335		
gac	atc	tgg	ggc	cag	ggc	acc	ctg	gtc	acc	gtg	tcc	tca	gcc	agc	acc	1056
Asp	Ile	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	
			340				345						350			
aag	ggg	ccc	tct	gtg	ttt	ccc	ctt	gcc	cct	tgc	agt	agg	tct	acc	agc	1104
Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	
		355					360					365				
gaa	agt	act	gcc	gcc	ctt	ggc	tgt	ctg	gtg	aaa	gat	tat	ttt	cct	gaa	1152
Glu	Ser	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	
	370					375					380					

cct gtc acc gtg tcc tgg aac tcc ggt gct ttg act tct ggc gtt cat Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 385 390 395 400	1200
act ttt cct gca gtc ctg caa agt tct ggc ctg tac agc ctt agc tcc Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 405 410 415	1248
gtg gta act gtg cct tcc tct tct ctg ggt acc aaa acc tat acc tgc Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys 420 425 430	1296
aat gtg gac cac aaa cct tct aat act aag gtc gac aag agg gtg gag Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu 435 440 445	1344
tct aag tac gga cca cct tgt cct cca tgc ccc gcc ccc gag ttc gag Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu 450 455 460	1392
ggc ggt cct agt gtg ttc ctg ttc cct cca aag ccc aag gac acc ttg Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu 465 470 475 480	1440
atg ata agc agg act cct gag gtg aca tgt gtg gtt gta gac gtc tct Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser 485 490 495	1488
cag gag gat ccc gaa gtg cag ttt aat tgg tac gtg gat gga gtc gag Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu 500 505 510	1536
gtg cac aac gcc aaa acc aaa ccc cgc gag gag caa ttc aac tcc acc Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr 515 520 525	1584
tat cgc gtg gtg tct gtc ctg acc gtc ctg cac caa gat tgg ctg aac Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn 530 535 540	1632
gga aaa gaa tat aag tgt aaa gta agc aat aag ggc ctg cct tca tcc Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser 545 550 555 560	1680
att gag aag aca atc agc aag gca aag ggc cag cct aga gaa ccc caa Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln 565 570 575	1728
gtg tac acc ctc cca ccc tct cag gag gaa atg acc aag aat cag gtt Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val 580 585 590	1776
agc ctt act tgt ctc gta aag ggg ttc tac cct agc gac att gct gtc Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 595 600 605	1824

gag tgg gaa agc aat gga cag cct gag aat aac tat aaa acc act ccc	1872
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro	
610 615 620	
cca gtg ctt gac tca gat ggc tct ttt ttc ctt tac tcc cgc ttg aca	1920
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr	
625 630 635 640	
gtc gac aag agt aga tgg caa gag ggg aac gtg ttc agc tgc agt gtt	1968
Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val	
645 650 655	
atg cac gag gca ctc cat aac cat tat act cag aaa tcc ttg agc ctg	2016
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu	
660 665 670	
tcc ctt gga aag	2028
Ser Leu Gly Lys	
675	

<210> 10
 <211> 676
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 10

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
225 230 235 240

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
245 250 255

Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
260 265 270

Glu Trp Val Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala
275 280 285

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
290 295 300

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
305 310 315 320

Tyr Tyr Cys Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe
325 330 335

Asp Ile Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
340 345 350

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
355 360 365

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
370 375 380

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
385 390 395 400

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
405 410 415

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys
420 425 430

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
435 440 445

Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu
450 455 460

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
465 470 475 480

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
485 490 495

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
500 505 510

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
515 520 525

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
530 535 540

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
545 550 555 560

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
565 570 575

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
580 585 590

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
595 600 605

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
610 615 620

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
625 630 635 640

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
645 650 655

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
660 665 670

Ser Leu Gly Lys
675

<210> 11
<211> 1356
<212> DNA
<213> Artificial Sequence

<220>
<223> H-chain of anti-human Tie2 antibody

<220>
<221> CDS
<222> (1).. (1356)

<400> 11	
gag gtg cag ctg gtg gaa tcc ggc gga ggc ctg gtg cag cct ggc ggc	48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
tct ctg aga ctg tcc tgt gcc gcc tcc ggc ttc acc ttc gac gac tac	96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr	
20 25 30	
gcc atg cac tgg gtc cga cag gcc cct ggc aag ggc ctg gaa tgg gtg	144
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	

gcc ggc atc tcc tgg aac tcc ggc tcc atc gtg tac gcc gac tcc gtg Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val 50 55 60	192
aag ggc cgg ttc acc atc tcc cgg gac aac tcc aag aac acc ctg tac Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80	240
ctg cag atg aac tcc ctg cgg gcc gag gac acc gcc gtg tac tac tgc Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95	288
gcc aag gac atc aga gag cag ctg gtc gag gac gcc ttc gac atc tgg Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp 100 105 110	336
ggc cag ggc acc ctg gtc acc gtg tcc tca gcc tcc acc aag ggc cca Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro 115 120 125	384
tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr 130 135 140	432
gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr 145 150 155 160	480
gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro 165 170 175	528
gct gtc cta cag tcc tca gga ctc tac tcc ott agt agc gtg gtg acc Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr 180 185 190	576
gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn 195 200 205	624
cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc aaa tct His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser 210 215 220	672
tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu 225 230 235 240	720
ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu 245 250 255	768
atg atc tcc cgg acc oct gag gtc aca tgc gtg gtg gtg gac gtg agc Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser 260 265 270	816

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu 275 280 285	864
gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr 290 295 300	912
tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn 305 310 315 320	960
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro 325 330 335	1008
atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln 340 345 350	1056
gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val 355 360 365	1104
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 370 375 380	1152
gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro 385 390 395 400	1200
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr 405 410 415	1248
gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val 420 425 430	1296
atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu 435 440 445	1344
tct ccg ggt aaa Ser Pro Gly Lys 450	1356

<210> 12
 <211> 452
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 12

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Gly Ile Ser Trp Asn Ser Gly Ser Ile Val Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Ile Arg Glu Gln Leu Val Glu Asp Ala Phe Asp Ile Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445

Ser Pro Gly Lys
450

<210> 13
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 13

Glu Pro Lys Ser Cys Gly Ser
1 5

<210> 14
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 14

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Ser
1 5 10

<210> 15
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 15

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Gly Gly Gly
1 5 10 15

Ser Gly Gly Gly Gly Ser
20

<210> 16
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 16

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Gly Gly Gly
1 5 10 15

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

<210> 17

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 17

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Gly Gly Gly
1 5 10 15

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
35 40

<210> 18

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 18

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Gly Gly Gly
1 5 10 15

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
35 40 45

Gly Gly Gly Ser
50

<210> 19
<211> 62
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 19

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Gly Gly Gly Gly
1 5 10 15

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
35 40 45

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
50 55 60

<210> 20
<211> 64
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 20

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Ser Pro Arg Ser
1 5 10 15

Pro Glu Pro Lys Ser Ser Asp Thr Pro Pro Pro Ser Pro Arg Ser Pro
20 25 30

Glu Pro Lys Ser Ser Asp Thr Pro Pro Pro Ser Pro Arg Ser Pro Glu
35 40 45

Pro Lys Ser Ser Asp Thr Pro Pro Pro Ser Pro Arg Ser Pro Gly Ser
50 55 60

<210> 21
<211> 3369
<212> DNA

<213> Homo sapiens

<400> 21

atggactcctt tagccagcctt agttctctgt ggagtcagct tgctcctttc tggaactgtg	60
gaaggtgccca tggacttgat cttgatcaat tccctacctc ttgtatctga tgctgaaaca	120
tctctcacct gcattgcctc tgggtggcgc ccccatgagc ccatcaccat aggaagggac	180
tttgaagcct taatgaacca gcaccaggat ccgctggaag ttactcaaga tgtgaccaga	240
gaatgggcta aaaaagttgt ttggaagaga gaaaaggcta gtaagatcaa tgggtgcttat	300
ttctgtgaag ggcgagttcg aggagaggca atcaggatac gaaccatgaa gatgcgtcaa	360
caagcttcct tcctaccagc tactttaact atgactgtgg acaagggaga taactgtaac	420
atatctttca aaaaggtatt gactaaagaa gaagatgcag tgatttaca aaatggttcc	480
ttcatccatt cagtgcoccg gcatgaagta cctgatattc tagaagtaca cctgcctcat	540
gctcagcccc aggatgotgg agtgtactcg gccaggata taggaggaaa cctcttcacc	600
tgggccttca ccaggotgat agtccggaga tgtgaagccc agaagtgggg acctgaatgc	660
aaccatctct gtactgottg tatgaacaat ggtgtctgcc atgaagatac tggagaatgc	720
atttgccctc ctgggtttat gggaaggacg tgtgagaagg cttgtgaact gcacacgttt	780
ggcagaactt gtaaagaaag gtgcagtgga caagagggat gcaagtctta tgtgttctgt	840
ctccctgacc cctatgggtg ttctgtgcc acaggctgga agggctctgca gtgcaatgaa	900
gcatgccacc ctggttttta cgggccagat tgtaagctta ggtgcagctg caacaatggg	960
gagatgtgtg atcgcttcca aggatgtctc tgctctccag gatggcaggg gctccagtgt	1020
gagagagaag gcataccgag gatgacccca aagatagtgg atttgccaga tcatatagaa	1080
gtaaacagtg gtaaatttaa tcccatttgc aaagcttctg gctggccgct acctactaat	1140
gaagaaatga ccctggtgaa gccggatggg acagtgtcc atccaaaaga ctttaaccat	1200
acggatcatt tctcagtagc catattcacc atccaccgga tcctcccccc tgactcagga	1260
gtttgggtct gcagtgtgaa cacagtggct gggatgggtg aaaagccctt caacatttct	1320
gttaaagttc ttccaaagcc cctgaatgcc ccaaactga ttgacactgg acataacttt	1380
gctgtcatca acatcagctc tgagccttac tttggggatg gaccaatcaa atccaagaag	1440
cttctataca aaccogttaa tcaactatgag gcttggcaac atattcaagt gacaaatgag	1500
attgttiacac tcaactatit ggaacctcgg acagaatatg aactctgtgt gcaactggtc	1560
cgtcgtggag aggggtgggga agggcatcct ggacctgtga gacgcttcac aacagcttct	1620

atcggactcc ctcctccaag aggtctaaat ctcctgccta aaagtcagac cactctaaat	1680
ttgacctggc aaccaatatt tccaagctcg gaagatgact tttatgttga agtggagaga	1740
aggtctgtgc aaaaaagtga tcagcagaat attaaagttc caggcaactt gacttcggtg	1800
ctacttaaca acttacatcc cagggagcag tacgtggtcc gagctagagt caacaccaag	1860
gcccaggggg aatggagtga agatctcact gcttggaccc ttagtgacat tcttcctcct	1920
caaccagaaa acatcaagat ttccaacatt acacactcct cagctgtgat ttcttggaca	1980
atattggatg gctattctat ttcttctatt actatccgtt acaaggttca aggcaagaat	2040
gaagaccagc acgttgatgt gaagataaag aatgccacca tcaactcagta tcagctcaag	2100
ggcctagagc ctgaaacagc ataccaggtg gacatTTTTg cagagaacaa catagggtca	2160
agcaaccag ctttttctca tgaactggtg accctcccag aatctcaagc accagcggac	2220
ctcggagggg ggaagatgct gcttatagcc atccttggct ctgctggaat gacctgcctg	2280
actgtgctgt tggcctttct gatcatattg caattgaaga gggcaaagt gcaaaggaga	2340
atggoccaaag ctttccaaaa cagggaagaa ccagctgtgc agttcaactc agggactctg	2400
gocctaaaca ggaaggtcaa aaacaacca gatcctacaa tttatccagt gcttgactgg	2460
aatgacatca aatttcaaga tgtgattggg gagggcaatt ttggccaagt tcttaaggcg	2520
cgcatcaaga aggatgggtt acggatggat gctgccatca aaagaatgaa agaatatgcc	2580
tccaaagatg atcacaggga ctttgcagga gaactggaag ttctttgtaa acttggacac	2640
catccaaaca tcatcaatct cttaggagca tgtgaacatc gaggtactt gtacctggcc	2700
attgagtacg cgcccatgg aaaccttctg gacttccttc gcaagagccg tgtgctggag	2760
acggaccag catttgccat tgccaatagc accgcgtcca cactgtcctc ccagcagctc	2820
cttcacttcg ctgccgacgt ggccgggggc atggactact tgagccaaaa acagtttato	2880
cacagggatc tggctgccag aaacatttta gttggtgaaa actatgtggc aaaaatagca	2940
gattttggat tgtcccgagg tcaagagggt tatgtgaaaa agacaatggg aaggctccca	3000
gtgcgctgga tggccatcga gtcactgaat tacagtgtgt acacaacca cagtgatgta	3060
tggctctatg gtgtgttact atgggagatt gttagcttag gaggcacacc ctactgcgga	3120
atgacttgtg cagaactcta cgagaagctg cccagggct acagactgga gaagcccctg	3180
aactgtgatg atgagggtga tgatctaata agacaatgct ggcgggagaa gccttatgag	3240
aggccatcat ttgccagat attggtgtcc ttaaacagaa tgttagagga gcgaaagacc	3300
tacgtgaata ccacgcttta tgagaagttt acttatgcag gaattgactg ttctgctgaa	3360

gaagcggcc

3369

<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> thrombin cleavage site

<400> 22

Leu Val Pro Arg Gly Ser
1 5

<210> 23
<211> 231
<212> PRT
<213> Homo sapiens

<400> 23

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
1 5 10 15

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
20 25 30

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
35 40 45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65 70 75 80

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
85 90 95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
100 105 110

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
115 120 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
130 135 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
165 170 175

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
180 185 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
195 200 205

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
210 215 220

Leu Ser Leu Ser Pro Gly Lys
225 230