

**(12) STANDARD PATENT**  
**(19) AUSTRALIAN PATENT OFFICE**

(11) Application No. **AU 2017273959 B2**

(54) Title  
**ETAR antibody, pharmaceutical composition and use thereof**

(51) International Patent Classification(s)  
**C07K 16/28** (2006.01) **A61P 11/00** (2006.01)  
**A61K 39/395** (2006.01) **A61P 35/00** (2006.01)  
**A61P 9/12** (2006.01) **C12N 15/13** (2006.01)

(21) Application No: **2017273959** (22) Date of Filing: **2017.05.27**

(87) WIPO No: **WO17/206840**

(30) Priority Data

(31) Number	(32) Date	(33) Country
<b>201610376600.7</b>	<b>2016.05.31</b>	<b>CN</b>
<b>201610954533.2</b>	<b>2016.10.27</b>	<b>CN</b>

(43) Publication Date: **2017.12.07**

(44) Accepted Journal Date: **2024.08.01**

(71) Applicant(s)  
**Gmax Biopharm LLC**

(72) Inventor(s)  
**Zhang, Cheng;Fan, Kesuo;Guo, Yong;Yao, Chenjiang;Zhang, Hua;Wang, Xiaofeng;Jing, Shuqian**

(74) Agent / Attorney  
**FB Rice Pty Ltd, L 23 44 Market St, Sydney, NSW, 2000, AU**

(56) Related Art  
**JP 2008280266 A**

# (12) 按照专利合作条约所公布的国际申请

(19) 世界知识产权组织  
国际局



(43) 国际公布日  
2017 年 12 月 7 日 (07.12.2017)

WIPO | PCT

(10) 国际公布号

WO 2017/206840 A1

## (51) 国际专利分类号:

C07K 16/28 (2006.01) A61P 9/12 (2006.01)

C12N 15/13 (2006.01) A61P 11/00 (2006.01)

A61K 39/395 (2006.01) A61P 35/00 (2006.01)

(21) 国际申请号: PCT/CN2017/086369

(22) 国际申请日: 2017 年 5 月 27 日 (27.05.2017)

(25) 申请语言: 中文

(26) 公布语言: 中文

## (30) 优先权:

201610376600.7 2016 年 5 月 31 日 (31.05.2016) CN

201610954533.2 2016 年 10 月 27 日 (27.10.2016) CN

(71) 申请人: 杭州鸿运华宁生物医药工程有限公司 (GMAX BIOPHARM LLC) [CN/CN]; 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。

(72) 发明人: 张成(ZHANG, Cheng); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 范克索(FAN, Kesuo); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 郭勇(GUO, Yong); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 药晨江(YAO, Chenjiang); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 章华(ZHANG, Hua); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 汪笑峰(WANG, Xiaofeng); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。 景书谦(JING, Shuqian); 中国浙江省杭州市滨江区秋溢路 288 号东冠高新科技园 2 号楼三层, Zhejiang 310052 (CN)。

(74) 代理人: 北京安信方达知识产权代理有限公司(AFD CHINA INTELLECTUAL PROPERTY LAW OFFICE); 中国北京市海淀区学清路 8 号 B 座 1601A, Beijing 100192 (CN)。

(81) 指定国(除另有指明, 要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG,

BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW。

(84) 指定国(除另有指明, 要求每一种可提供的地区保护): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 欧亚 (AM, AZ, BY, KG, KZ, RU, TJ, TM), 欧洲 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG)。

## 本国际公布:

- 包括国际检索报告(条约第 21 条(3))。
- 包括说明书序列表部分(细则 5.2(a))。

(54) Title: ET<sub>A</sub>R ANTIBODY, PHARMACEUTICAL COMPOSITION AND USE THEREOF

(54) 发明名称: ET<sub>A</sub>R 抗体, 其药物组合物及其应用

(57) Abstract: Disclosed is a stable solution preparation of an ET<sub>A</sub>R antibody for treating, preventing or alleviating one or more symptoms of pulmonary hypertension and one or more symptoms of genital cancer.

(57) 摘要: ET<sub>A</sub>R 抗体的稳定溶液制剂, 用于治疗、预防或改善肺动脉高血压的一种或多种症状以及生殖器官癌症的一种或多种症状。



WO 2017/206840 A1

# ET<sub>A</sub>R ANTIBODY, AND PHARMACEUTICAL COMPOSITIONS AND USE THEREOF

## FIELD

Provided herein are an ET<sub>A</sub>R antibody and a pharmaceutical composition thereof, for example, a stable pharmaceutical solution formulation of the ETAR antibody. Also provided herein is a method of treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension or one or more symptoms of cancer of a reproductive organ.

## BACKGROUND

Endothelin (ET) is a vasoconstriction peptide hormone, important to the homeostasis and regulation of the biological functions of the cardiovascular system. ET is found not only in the endothelium but also in many other tissues and cell types (Barton *et al.*, 2008, *Can. J. Physiol. Pharmacol.* 86:485-498). ET is a 2,400 Da peptide of 21 amino acids, having 2 disulfide bonds at its N-terminus, linking the 1st and 15th cysteine residues and the 3rd and 11th cysteine residues, respectively. Its C-terminus contains hydrophobic amino acid residues. Its N-terminal structure is important for binding to its receptor, while its C-terminal structure is important as to where on the receptor to bind. ET has three isoforms: ET-1, ET-2 and ET-3. They differ by a few amino acid residues. ET-1 plays a major role in the regulation of the biological functions of the cardiovascular system. Upon stimulation, endothelial cells synthesize and release ET-1. ET-1 is mainly regulated at the transcription level.

Endothelin receptors (ETR) has two isoforms: ET<sub>A</sub>R and ET<sub>B</sub>R, which belong to the G protein-coupled receptor (GPCR) family. Upon stimulation, ET<sub>A</sub>R activates membrane Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) and Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE) to increase cellular Ca<sup>2+</sup> concentrations and to sensitize muscle fibers to Ca<sup>2+</sup>, resulting in the constriction of vascular smooth muscle and cardiac muscle (Neylon, 1999, *Clin. Exp. Pharmacol. Physiol.* 26:149-153). Unlike ET<sub>A</sub>R, ET<sub>B</sub>R mainly relaxes the vascular smooth muscle cells and cardiac muscle cells (Nelson *et al.*, 2003, *Nat. Rev. Cancer* 3:110-113).

ET<sub>A</sub>R belongs to GPCR family A and has seven transmembrane domains. The extracellular domain is short and small, and only accounts for about one seventh of the full-length receptor, while a GPCR antibody can only target its extracellular domain.

Therefore, the structural feature and naturally low expression level of a GPCR make it difficult to produce biologically active antigens.

Pulmonary arterial hypertension (PAH) is due to the vasoconstriction of the lung or lung related vasculature, resulting in lung artery insufficiency and a compensatory increase in the blood pressure of the heart. On the microscopic scale, there appear to be changes in the small pulmonary arteries, including intimal fibrosis, medial hypertrophy, and plexiform lesions, causing *in situ* thrombosis of elastic and small pulmonary arteries, and resulting in increased blood circulation resistance in the whole lung vasculature (Simonneau *et al.*, 2004, *J. Am. Coll. Cardiol.* 43:5S–12S; Barst *et al.*, 2004, *J. Am. Coll. Cardiol.* 43:40S–47S). PAH is a disease with a fairly high rate of disability or death. It is a devastating disease that severely affects the health of patients and imposes significant burden on society.

The severity of PAH depends on the degree of relevant cardiac deformity, and the common congenital cardiac abnormalities that will result in secondary PAH includes: aortic stenosis, aortopulmonary window, atrial septal defect, complete atrioventricular septal defect, artery coarctation, dilated cardiomyopathy, double outlet right ventricle, hypertrophic cardiomyopathy, mitral stenosis, patent ductus arteriosus, single ventricle, persistent truncus arteriosus, and ventricular septal defect. PAH mainly affects pulmonary arteries and right heart, causing right ventricular hypertrophy, right atrial dilatation, the dilatation of the trunk of the pulmonary artery, and the sparsity of the surrounding pulmonary arterioles. The hypertrophy of endothelial and smooth muscle cells of pulmonary arteriole results in tunica intima fibrosis, tunica media hypertrophy, lumina stenosis, occlusion or distortion, and plexus change. Tunica intima fibrosis and lumina occlusion may also afflict the pulmonary venules.

It has been shown that an ET<sub>A</sub>R antagonist can effectively block the increase in vascular pressure caused by endothelin to ameliorate PAH symptoms and improve exercise capability and hemodynamics in PAH patients (Serasli *et al.*, 2010, *Recent Pat. Cardiovasc. Drug Discov.* 5:184-95). The antibody provided herein can specifically bind to a human ET<sub>A</sub>R and attenuate pulmonary arterial pressure in an animal model. It can significantly improve a symptom of PAH in an animal model.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

## SUMMARY

In one aspect, the present disclosure provides a stable pharmaceutical solution formulation of an ETAR antibody, wherein the formulation comprises an ETAR antibody, a buffer, a surfactant and an amino acid protectant, wherein the formulation has a pH ranging from 5 to 7,

and wherein:

the ETAR antibody comprises light chain CDR1 amino acid sequence: SEQ ID NO: 8; light chain CDR2 amino acid sequence: SEQ ID NO: 32; light chain CDR3 amino acid sequence: SEQ ID NO: 50; heavy chain CDR1 amino acid sequence: SEQ ID NO: 70; heavy chain CDR2 amino acid sequence: SEQ ID NO: 92; and heavy chain CDR3 amino acid sequence: SEQ ID NO: 116; and

the ETAR antibody further comprises light chain constant domain amino acid sequence SEQ ID NO: 194 or SEQ ID NO: 196; and heavy chain constant domain amino acid sequence SEQ ID NO: 198; and

wherein the formulation further comprises a surfactant, and an amino acid protectant; wherein the concentration of the ETAR antibody is from 10 to 200 mg/mL; the concentration of the surfactant is from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is from 10 to 200 mM; and the concentration of the buffer is from 1 to 50 mM,

wherein the amino acid protectant comprises arginine hydrochloride,

wherein the buffer comprises sodium citrate, and

wherein the surfactant comprises polysorbate.

In a related aspect, the present disclosure provides a method of:

(a) treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension in a subject;

(b) treating, preventing, or alleviating one or more symptoms of a disease associated with elevated pulmonary arterial pressure in a subject; or

(c) treating, preventing, or alleviating one or more symptoms of cancer of a reproductive organ in a subject;

comprising administering to the subject a therapeutically effective amount of the formulation of the aforementioned aspect.

In a further related aspect, the present disclosure relates to the use of the formulation of the aforementioned aspect in the manufacture of a medicament for:

(a) treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension in a subject;

(b) treating, preventing, or alleviating one or more symptoms of a disease associated

with elevated pulmonary arterial pressure in a subject; or

(c) treating, preventing, or alleviating one or more symptoms of cancer of a reproductive organ in a subject.

Provided herein are an ET<sub>A</sub>R antibody and a pharmaceutical composition thereof, for

example, a stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody. Also provided herein is a method of treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension or one or more symptoms of cancer of a reproductive organ.

The ET<sub>A</sub>R antibody provided herein comprises 1, 2, 3, 4, 5, or 6 amino acid sequences, wherein each amino acid sequence is independently selected from:

- a . light chain CDR1 amino acid sequences: SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30;
- b . light chain CDR2 amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48;
- c . light chain CDR3 amino acid sequences: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68;
- d . heavy chain CDR1 amino acid sequences: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90;
- e . heavy chain CDR2 amino acid sequences: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ ID NO: 114; and
- f. heavy chain CDR3 amino acid sequences: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

Also provided herein is a pharmaceutical composition comprising an ET<sub>A</sub>R antibody provided herein and one or more pharmaceutically acceptable carriers.

Provided herein is a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody, comprising an ET<sub>A</sub>R antibody provided herein and a buffer.

Provided herein is a method of treating, preventing or alleviating one or more symptoms of pulmonary arterial hypertension in a subject, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition provided herein, for example, a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody provided herein.

Provided herein is a method of treating, preventing, or alleviating one or more symptoms of a disease associated with elevated pulmonary arterial pressure in a subject, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition provided herein, for example, a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody provided herein.

Provided herein is a method of treating, preventing, or alleviating one or more symptoms of cancer of a reproductive organ in a subject, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition provided herein, for example, a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody provided herein.

Provided herein is a kit for treating pulmonary arterial hypertension, a disease associated with elevated pulmonary arterial pressure, or cancer of a reproductive organ, comprising a pharmaceutical composition provided herein.

Provided herein is use of a pharmaceutical composition provided herein in the manufacture of a medicament for treating pulmonary arterial hypertension, a disease associated with elevated pulmonary arterial pressure, or cancer of a reproductive organ.

Provided herein is an isolated nucleic acid comprising a polynucleotide sequence encoding an ET<sub>A</sub>R antibody provided herein.

Provided herein is a recombinant expression vector comprising a nucleic acid provided herein.

Provided herein is a host cell comprising a vector provided herein.

Provided herein is a method for producing an ET<sub>A</sub>R antibody, comprising cultivating a host cell under conditions suitable for expressing an antibody provided herein.

## **BRIEF DESCRIPTION OF THE FIGURES**



Figure 1 shows the ELISA screening results of the supernatants of hybridomas for binding to CHO-DHFR-ET<sub>A</sub>R cells (labeled as CHO-ET<sub>A</sub>R in the figure). Among them, the ET<sub>A</sub>R antibody A-1 (comprising SEQ ID NO: 138 and SEQ ID NO: 166) was obtained from hybridoma clone 15F3.

Figure 2 shows the specific binding of recombinant ET<sub>A</sub>R antibodies (A-1, A-2 (comprising SEQ ID NO: 140 and SEQ ID NO: 168), A-7 (comprising SEQ ID NO: 150 and SEQ ID NO: 178), and A-12 (comprising SEQ ID NO: 160 and SEQ ID NO: 188)) to human ET<sub>A</sub>R as determined by FACS. The gray peak and the dotted peak are negative controls, the dotted peak representing the binding curve of the ET<sub>A</sub>R antibody to CHO-DHFR- and the solid line peak representing the binding curve of the ET<sub>A</sub>R antibody to CHO-DHFR-ET<sub>A</sub>R.

Figure 3 shows the inhibitory effects of the supernatants of hybridomas on cellular ET<sub>A</sub>R-mediated Ca<sup>2+</sup> changes as determined using a calcium flux assay.

Figure 4 shows the dose responses of recombinant ET<sub>A</sub>R antibodies on the inhibition of human ET<sub>A</sub>R as determined using a calcium flux assay (IC<sub>50</sub> = 37.91 nM, R<sup>2</sup> = 0.97) (A-1); (IC<sub>50</sub> = 87.84 nM, R<sup>2</sup> = 0.97) (A-9 (comprising SEQ ID NO: 154 and SEQ ID NO: 182)).

Figure 5 shows the *in vivo* activity of the recombinant ET<sub>A</sub>R (A-1) in a hypoxia-induced PAH cynomolgus monkey model. A-1 was found to be able to reduce the hypoxia-induced pulmonary systolic pressure significantly, and also to be effective within 96-hr as measured by area under the curve of the pulmonary systolic pressure *versus* time.

Figure 6 shows that the biological activity of ET<sub>A</sub>R antibody A-1 (25 mg/mL) did not change significantly after 3 months of storage in a formulation solution containing 20 mM sodium citrate, 140 mM arginine-HCl, and 0.04% TWEEN-80 at pH 5.8 and 4 °C.

Figure 7 shows that the biological activity of ET<sub>A</sub>R antibody A-1 (25 mg/mL) did not change significantly after 3 months of storage in a formulation solution containing 20 mM sodium citrate, 140 mM arginine-HCl, and 0.04% TWEEN-80 at pH 5.8 and 25 °C.

## **DETAILED DESCRIPTION**

### **Definitions**

Unless otherwise defined herein, scientific and technical terms provided herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclatures and techniques provided herein in connection with pharmacology, biology, biochemistry, cell and tissue culture, molecular biology, immunology, microbiology,

genetics and protein and nucleic acid chemistry and hybridization are those well-known and commonly used in the art.

Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The standard one- or three-letter abbreviations provided herein describe polynucleotide and polypeptide sequences. Unless otherwise specified, polypeptide sequences have their amino termini at the left and their carboxyl termini at the right, and single-stranded nucleic acid sequences and the top strands of double-stranded nucleic acid sequences have their 5' termini at the left and their 3' termini at the right. A particular section of a polypeptide can be designated by amino acid residue numbers such as amino acids 80 to 130, or in combination with the corresponding actual residues such as Lys80 to Lys130. A particular polypeptide or polynucleotide sequence also can be described by showing its differences from a reference sequence.

The terms “peptide” “polypeptide” and “protein” each refer to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, *e.g.*, native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

The term “polypeptide fragment” refers to a polypeptide that has an amino-terminal and/or carboxyl-terminal deletion as compared to a corresponding full-length protein. Fragments can be, for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 50, 70, 80, 90, 100, 150 or 200 amino acids in length. Fragments can also be, for example, at most 1,000, 750, 500, 250, 200, 175, 150, 125, 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 14, 13, 12, 11, or 10 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (*e.g.*, an Fc or leucine zipper domain) or an artificial amino acid sequence (*e.g.*, an artificial linker sequence).

Polypeptides of the disclosure include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter susceptibility to form a protein complex, (4) alter binding

affinities, and (4) confer or modify other physicochemical or functional properties. Analogs include muteins of a polypeptide. For example, single or multiple amino acid substitutions

(*e.g.*, conservative amino acid substitutions) can be made in the naturally occurring sequence (*e.g.*, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A “conservative amino acid substitution” is one that does not substantially change the structural characteristics of the parent sequence (*e.g.*, replacement of an amino acid should not break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterize the parent sequence or are necessary for its functionality).

A “variant” of a polypeptide comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants of the disclosure include fusion proteins.

A “derivative” of a polypeptide is a polypeptide that has been chemically modified, *e.g.*, *via* conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (*e.g.*, human serum albumin), phosphorylation, and glycosylation.

Unless otherwise indicated, the term “antibody” includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

An “antibody” is a protein comprising a portion that binds to an antigen and optionally a scaffold or framework portion that allows the antibody to adopt a conformation that promotes the binding of the antibody to the antigen. Examples of antibodies include antibodies, antibody fragments (*e.g.*, an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antibody can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDRs derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced, for example, to stabilize the three-dimensional structure of the antibody as well as completely synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer *et al.*, 2003, *Proteins: Structure, Function, and Bioinformatics*, 53:121-129; Roque *et al.*, 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics (“PAMs”) can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

An antibody can have, for example, the structure of a naturally occurring

immunoglobulin. An “immunoglobulin” is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxyl terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody’s isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a “J” region of about 12 or more amino acids. The heavy chain also includes a “D” region of about 10 more amino acids. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated herein by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each region is in accordance with the definitions of Kabat *et al.* in *Sequences of Proteins of Immunological Interest*, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication No. 91-3242, 1991.

Unless otherwise specified, an “antibody” refers to an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding. Antigen binding portions can be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, *inter alia*, Fab, Fab', F(ab')<sub>2</sub>, Fv, domain antibodies (dAbs), fragments including complementarity determining regions (CDRs), single-chain antibodies (scFv), chimeric antibodies, diabodies, tribodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

A Fab fragment is a monovalent fragment having the V<sub>L</sub>, V<sub>H</sub>, C<sub>L</sub> and C<sub>H1</sub> regions; a F(ab')<sub>2</sub> fragment is a bivalent fragment having two Fab fragments linked by a disulfide

bridge at the hinge region; a Fd fragment has the V<sub>H</sub> and C<sub>H1</sub> regions; and a dAb fragment has a V<sub>H</sub> region, a V<sub>L</sub> region, or an antigen-binding fragment of a V<sub>H</sub> or V<sub>L</sub> region (US. Pat. Nos. 6846634, 6696245, US App. Pub. Nos. 05/0202512, 04/0202995, 04/0038291, 04/0009507, 03/0039958, Ward et al., 1989, *Nature* 341:544-546).

A single-chain antibody (scFv) is an antibody in which a V<sub>L</sub> and a V<sub>H</sub> region are joined via a linker (*e.g.*, a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (*see, e.g.*, Bird *et al.*, 1988, *Science* 242:423-26 and Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V<sub>H</sub> and V<sub>L</sub> regions joined by a linker that is too short to allow for pairing between two regions on the same chain, thus allowing each region to pair with a complementary region on another polypeptide chain (*see, e.g.*, Holliger *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-48, and Poljak *et al.*, 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

Complementarity determining regions (CDRs) and frame work regions (FR) of a given antibody can be identified using the system described by Kabat *et al.* in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. One or more CDRs can be incorporated into a molecule either covalently or noncovalently to make it an antibody. An antibody can incorporate the CDR(s) as part of a larger polypeptide chain, can covalently link the CDR(s) to another polypeptide chain, or can incorporate the CDR(s) noncovalently. The CDRs permit the antibody to specifically bind to a particular antigen of interest.

An antibody can have one or more binding sites. If there are more than one binding sites, the binding sites can be identical to one another or can be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a “bispecific” or “bifunctional” antibody has two different binding sites.

The term “murine antibody” includes all antibodies that have one or more variable and constant regions derived from a murine immunoglobulin sequence.

The term “humanized antibody” refers to an antibody that produced by grafting the complementarity determining region sequence of a murine antibody molecule into a human antibody variable region framework.

The term “antigen-binding domain,” “antigen-binding region,” or “antibody-binding site” is a portion of an antibody that comprises amino acid residues (or other portion) interacting with an antigen and contributing to the specificity and affinity of the antibody for the antigen. For antibodies that specifically bind to their antigen, this will include at least a portion of at least one of its CDR regions.

The term “epitope” is the portion of a molecule that is bound by an antibody (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide’s primary sequence but that, in the context of the polypeptide’s tertiary and quaternary structure, are near enough to each other to be bound by an antibody).

The “percent identity” of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

The terms “polynucleotide,” “oligonucleotide” and “nucleic acid” are used interchangeably throughout and include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs (*e.g.*, peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody of the invention, or a fragment, derivative, mutein, or variant thereof.

Two single-stranded polynucleotides are “the complement” of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps and without unpaired nucleotides at the 5' or the 3' end of either

sequences. A polynucleotide is “complementary” to another polynucleotide if the two polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a polynucleotide can be complementary to another polynucleotide without being its complement.

The term “vector” is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a “plasmid,” which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An “expression vector” is a type of vectors that can direct the expression of a chosen polynucleotide.

A nucleotide sequence is “operably linked” to a regulatory sequence if the regulatory sequence affects the expression (*e.g.*, the level, timing, or location of expression) of the nucleotide sequence. A “regulatory sequence” is a nucleic acid that affects the expression (*e.g.*, the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (*e.g.*, polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, *Gene Expression Technology: Methods in EnZymology* 185, Academic Press, San Diego, Calif. and Baron *et al.*, 1995, *Nucleic Acids Res.* 23:3605-06.

The term “host cell” is a cell that can be used to express a nucleic acid, *e.g.*, a nucleic acid of the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be an eukaryote, for example, a single-celled eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Typically, a host cell



is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase “recombinant host cell” can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, *e.g.*, mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

#### Endothelin receptor

Endothelin A receptor (ET<sub>A</sub>R) belongs to family A of 7-transmembrane receptors that are coupled to one or more intracellular signaling pathways *via* heterotrimeric guanine nucleotide-binding proteins (G proteins) (Jelinek *et al.*, 1993, *Science* 259:1614-1616, Segre *et al.*, 1993, *Trends Endocrinol. Metab.* 4:309-314). As used herein, “endothelin receptor” and “ET<sub>A</sub>R” are used interchangeably.

In one embodiment, the antibody provided herein can be selected to bind to membrane bound endothelin receptors as expressed on cells, and inhibit or block endothelin signaling through the endothelin receptors. In one embodiment, the antibody provided herein specifically binds to the human endothelin receptor. In a further embodiment, the antibody binding to the human endothelin receptor can also bind to the endothelin receptors of other species, *e.g.*, rat. The examples below provide one method of generating murine antibodies which bind to human membrane-bound endothelin receptors, and in a further embodiment, bind to endothelin receptors of other species.

The polynucleotide and polypeptide sequences for several species of the endothelin receptors are known. SEQ ID NO: 1-SEQ ID NO: 6 present sequences for human, monkey, and rat. The sequence data were obtained from the GeneBank database of the National Center for Biotechnology Information.

#### Endothelin A receptor:

Human (*Homo sapiens*) polynucleotides (SEQ ID NO: 1); accession number: S63938.

Human (*Homo sapiens*) amino acid (SEQ ID NO: 2); accession number: AAB20278.

Cynomolgus (*Homo sapiens*) polynucleotides (SEQ ID NO: 3); accession number: JV635771.

Cynomolgus (*Homo sapiens*) amino acid (SEQ ID NO: 4); accession number: AFJ71111.

Rat (*Rattus norvegicus*) polynucleotides (SEQ ID NO: 5); accession number: M60786.

Rat (*Rattus norvegicus*) amino acid (SEQ ID NO: 6); accession number: AAA41114.  
Endothelin receptor A (ET<sub>A</sub>R) antibody

In one embodiment, an ET<sub>A</sub>R antibody (for example, a full-length antibody, antibody fragment, antibody derivative, antibody variant, and antibody mutein) is provided herein.

In one embodiment, the ET<sub>A</sub>R antibody provided herein comprises 1, 2, 3, 4, 5, or 6 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR1 amino acid sequences: SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30;
- b . light chain CDR2 amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48;
- c . light chain CDR3 amino acid sequences: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68;
- d . heavy chain CDR1 amino acid sequences: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90;
- e . heavy chain CDR2 amino acid sequences: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ

ID NO: 114; and

f . heavy chain CDR3 amino acid sequences: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

Table 1 lists light chain CDR amino acid sequences of the ET<sub>A</sub>R antibodies provided herein, as well as their polynucleotides coding sequences. Table 2 lists heavy chain CDR amino acid sequences of the ET<sub>A</sub>R antibodies provided herein, as well as their polynucleotides coding sequences.

Table1: light chain CDR amino acid sequences and polynucleotide coding sequences

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
A-1 Nucleic Acid	agggccagtcagaacattggcaca agcatacac (SEQ ID NO: 7)	tatgcttctaagctatatct (SEQ ID NO: 31)	caacatagttatagctggccgt ggacg (SEQ ID NO: 49)
A-1 Amino Acid	RASQNIGTSIH (SEQ ID NO: 8)	YASKSIS (SEQ ID NO: 32)	QHSYSWPWT (SEQ ID NO: 205) or QHSYSFPWT (SEQ ID NO: 50)
A-2 Nucleic Acid	cgagcaagtgaaaatatttacagtta ttagca (SEQ ID NO: 9)	aatgcaaaaaccttagcaga a (SEQ ID NO: 33)	cagcatcattatggattccgtt cacg (SEQ ID NO: 51)
A-2 Amino Acid	RASENIYSYLA (SEQ ID NO: 10)	NAKTLAE (SEQ ID NO: 34)	QHGYGIPFT (SEQ ID NO: 52)
A-3 Nucleic Acid	cagagcctcttgatattgatggaaa gacatattgaat (SEQ ID NO: 11)	ctgggtgtctgaattggactct (SEQ ID NO: 35)	tggcaaggtacacattttccgc tcacg (SEQ ID NO: 53)
A-3 Amino Acid	QSLFDIDGKTYLN (SEQ ID NO: 12)	LVSELD (SEQ ID NO: 36)	WQGTHFPLT (SEQ ID NO: 54)
A-4 Nucleic Acid	cgggcaagtcaggacattggtgta gcttaaac (SEQ ID NO: 13)	gccacatccagcttagattct (SEQ ID NO: 37)	ctacaatatgctagttctccgta tacg (SEQ ID NO: 55)
A-4 Amino Acid	RASQDIGGSLN (SEQ ID NO: 14)	ATSSLDS (SEQ ID NO: 38)	LQYASSPYT (SEQ ID NO: 56)

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
A-5 Nucleic Acid	agggccagccagactattagcgact tcttacac (SEQ ID NO: 15)	tatgcttcccaatccatctct (SEQ ID NO: 39)	caaagtggtaacacctttccgt ggacg (SEQ ID NO: 57)
A-5 Amino Acid	RASQTISDFLH (SEQ ID NO: 16)	YASQSIG (SEQ ID NO: 40)	QSGNTFPWT (SEQ ID NO: 58)
A-6 Nucleic Acid	agggcaagtgaggacatacacact caattagcc (SEQ ID NO: 17)	ggtgcagccagtttgaag t (SEQ ID NO: 41)	caacagtataggagtattccgt ggacg (SEQ ID NO: 59)
A-6 Amino Acid	RASEDIHTQLA (SEQ ID NO: 18)	GAASLKS (SEQ ID NO: 42)	QQYRSIPWT (SEQ ID NO: 60)
A-7 Nucleic Acid	agatctagtcagtacattgtcatagt actggaaccacctatttagaa (SEQ ID NO: 19)	aaagttccaaccgattttct (SEQ ID NO: 43)	tttcaaggttcacattttccattc acg (SEQ ID NO: 61)
A-7 Amino Acid	RSSQYIVHSTGTTYLE (SEQ ID NO: 20)	KVSNRFS (SEQ ID NO: 44)	FQGSHFPFT (SEQ ID NO: 62)
A-8 Nucleic Acid	agatctagtcattacctgttcattgata acggaaacacctatgttgaa (SEQ ID NO: 21)	aaagttccaaccgattttct (SEQ ID NO: 43)	tttcaaggttcacattttccatt cacg (SEQ ID NO: 63)
A-8 Amino Acid	RSSHVLVHDNGNTYVE (SEQ ID NO: 22)	KVSNRFS (SEQ ID NO: 44)	FQGSHFPFT (SEQ ID NO: 62)
A-9 Nucleic Acid	agatctagtcagaacattgtccatag tactggaaacacctatttagaa (SEQ ID NO: 23)	aaagttccaaccgattttct (SEQ ID NO: 43)	tttcaaggttcacattttccattc acg (SEQ ID NO: 61)
A-9 Amino Acid	RSSQNIVHSTGNTYLE (SEQ ID NO: 24)	KVSNRFS (SEQ ID NO: 44)	FQGSHFPFT (SEQ ID NO: 62)
A-10 Nucleic Acid	agtgctcagctcaagtgttaagttacat acac (SEQ ID NO: 25)	gacacatccaaactggcttct (SEQ ID NO: 45)	caccagtggagtactaaccca cccacg (SEQ ID NO: 63)
A-10	SVSSSVSYIH	DTSKLAS	HQWSTNPPT

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
Amino Acid	(SEQ ID NO: 26)	(SEQ ID NO: 46)	(SEQ ID NO: 64)
A-11 Nucleic Acid	agtgccagctcaagtgttaagttacat gtgc (SEQ ID NO: 27)	gacacatccaaactggcttct (SEQ ID NO: 45)	cagcagtgaggagtagtaaccc accacg (SEQ ID NO: 65)
A-11 Amino Acid	SASSSVSYMC (SEQ ID NO: 28)	DTSKLAS (SEQ ID NO: 46)	QQWSSNPPT (SEQ ID NO: 66)
A-12 Nucleic Acid	cagggcattaacaattat (SEQ ID NO: 29)	tatacatcaactttacagtca (SEQ ID NO: 47)	cagcagtttagtaaacttcgga ca (SEQ ID NO: 67)
A-12 Amino Acid	QGINNY (SEQ ID NO: 30)	YTSTLQS (SEQ ID NO: 48)	QQFSKLRT (SEQ ID NO: 68)

Table2: heavy chain CDR amino acid sequences and polynucleotide coding sequences

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
A-1 Nucleic Acid	gggttctcactgaccactt ctggcttgggtgttgcc (SEQ ID NO: 69)	cacatttggcggatggtgacacgcg ctattaccagccctgaagaac (SEQ ID NO: 91)	atgaaggatgatagtcctta cttgacaac (SEQ ID NO: 115)
A-1 Amino Acid	GFSLTTSGLGVA (SEQ ID NO: 70)	HIWSDGDTRYYPALKN (SEQ ID NO: 92)	MKDDSLYFDN (SEQ ID NO: 116)
A-2 Nucleic Acid	ggctacacctttactagct actggatacac (SEQ ID NO: 71)	tacattaatcctgacactgattatagtg agtacaat (SEQ ID NO: 93)	gcaagtgctggttattatttt tttgacttc (SEQ ID NO: 117)
A-2 Amino Acid	GYTFTSYWIH (SEQ ID NO: 72)	YINPDTDYSEYN (SEQ ID NO: 94)	ASAGYYFFDF (SEQ ID NO: 118)
A-3 Nucleic Acid	ggcctcaacattaaagac atctatattcac (SEQ ID NO: 73)	aggattgatcctgcgaacggtaagac tgcatatgac (SEQ ID NO: 95)	ggtagggggggccac (SEQ ID NO: 119)
A-3 Amino Acid	GLNIKDIYIH (SEQ ID NO: 74)	RIDPANGKTAYD (SEQ ID NO: 96)	GRGAH (SEQ ID NO: 120)
A-4	ggttactcattcaccaact	atgattgatcctccgatgctgaaactg	gcaagaattggcgattact

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
Nucleic Acid	actggatacac (SEQ ID NO: 75)	gggttaa (SEQ ID NO: 97)	ataatatggactac (SEQ ID NO: 121)
A-4 Amino Acid	GYSFTNYWIH (SEQ ID NO: 76)	MIDPSDAETGLN (SEQ ID NO: 98)	ARIGDYYNMDY (SEQ ID NO: 122)
A-5 Nucleic Acid	ggattcactttcagtgacta tcccatgtct (SEQ ID NO: 77)	gttagtgatgggtggtccacc (SEQ ID NO: 99)	acaagacatgcttctacta tagctacgaccattctatgg actac (SEQ ID NO: 123)
A-5 Amino Acid	GFTFSDYPMS (SEQ ID NO: 78)	VSDGGGST (SEQ ID NO: 100)	TRHASYYSDHS MDY (SEQ ID NO: 124)
A-6 Nucleic Acid	ggattcactttcagtagctt tggcatgtct (SEQ ID NO: 79)	attagtagtgctgtagttcacc (SEQ ID NO: 101)	gcaagacgggggtacga cgttgggtgctttgaccac (SEQ ID NO: 125)
A-6 Amino Acid	GFTFSSFGMS (SEQ ID NO: 80)	ISSAGSFT (SEQ ID NO: 102)	ARRGYDVGCFD H (SEQ ID NO: 126)
A-7 Nucleic Acid	ggattcactttcagtaccta tggcatgtct (SEQ ID NO: 81)	accattaataactaatggtggtaccacct attatcgagacagtgtgaagggc (SEQ ID NO: 103)	gcaagagactacggggct atggactac (SEQ ID NO: 127)
A-7 Amino Acid	GFTFSTYGMS (SEQ ID NO: 82)	TINTNGGTTYRDSVKG (SEQ ID NO: 104)	ARDYGAMDY (SEQ ID NO: 128)
A-8 Nucleic Acid	ggattcactttcagtaccta tggcatgtct (SEQ ID NO: 81)	accataaataactaatggtggtaacacc tattattcagacaatgtgaagggc (SEQ ID NO: 105)	gcaagagactacggggct atggactac (SEQ ID NO: 127)
A-8 Amino Acid	GFTFSTYGMS (SEQ ID NO: 82)	TINTNGGNTYYSDNVKG (SEQ ID NO: 106)	ARDYGAMDY (SEQ ID NO: 128)
A-9 Nucleic Acid	ggattcactttcagtagtta tggcatgtct	accattagtagtaactggtgccaccgcc aattatccagacagtgtgaagggc	gcaactgaaaaggagct atgggctac

	<b>CDR1</b>	<b>CDR2</b>	<b>CDR3</b>
	(SEQ ID NO: 83)	(SEQ ID NO: 107)	(SEQ ID NO: 129)
A-9 Amino Acid	GFTFSSYGMS (SEQ ID NO: 84)	TISTNGATANYPDSVKG (SEQ ID NO: 108)	ATEKGAMGY (SEQ ID NO: 130)
A-10 Nucleic Acid	gggtttcactgaccacttc tggtatgggtgtaggc (SEQ ID NO: 85)	cacatttgggtgggatgatgataagtact ataatccatccctgaagagc (SEQ ID NO: 109)	gctcgaagaactgagact atgattacgacagtgtctata ttactatgctatggactac (SEQ ID NO: 131)
A-10 Amino Acid	GFSLTTSGLMGV G (SEQ ID NO: 86)	HIWWDDDKYYNPSLKS (SEQ ID NO: 110)	ARRTETMITTVL YYYAMDY (SEQ ID NO: 132)
A-11 Nucleic Acid	ggattttcactgagcacttc tggtttgggtgtaggc (SEQ ID NO: 87)	cacatttgggtgggatgatgataagtact ataatccatcccttaagaga (SEQ ID NO: 111)	gctcgaaggagggaagtt aacttcggtattaactattac tattctatggactac (SEQ ID NO: 133)
A-11 Amino Acid	GFSLSTSGGLGVG (SEQ ID NO: 88)	HIWWDDDKYYNPSLKR (SEQ ID NO: 112)	ARRREVNFGINY YYSMDY (SEQ ID NO: 134)
A-12 Nucleic Acid	ggattcaccttcagtgatta ttac (SEQ ID NO: 89)	attagaaatcgggctaattggttacaca aca (SEQ ID NO: 113)	gtaagagattcctatcacta cgggtacttcgatgtc (SEQ ID NO: 135)
A-12 Amino Acid	GFTFSDYY (SEQ ID NO: 90)	IRNRANGYTT (SEQ ID NO: 114)	VRDSYHYGYFD V (SEQ ID NO: 136)

In one embodiment, the antibody provided herein comprises a sequence that differs from a CDR sequence listed in Table 1 and Table 2 by 5, 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In another embodiment, the antibody provided herein comprises a sequence that differs from a CDR sequence listed in Table 1 and Table 2 by 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In yet another embodiment, the antibody provided herein comprises a sequence that differs from a CDR sequence listed

in Table 1 and Table 2 by 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In yet another embodiment, the antibody provided herein comprises a sequence that differs from a CDR sequence listed in Table 1 and Table 2 by 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In still another embodiment, the antibody provided herein comprises a sequence that differs from a CDR sequence listed in Table 1 and Table 2 by 1 amino acid addition, substitution, and/or deletion.

In another embodiment, the ET<sub>A</sub>R antibody provided herein (ET<sub>A</sub>R-1 antibody) comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR1 amino acid sequences: SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30; and
- b . heavy chain CDR1 amino acid sequences: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90.

In one aspect, the ET<sub>A</sub>R-1 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR2 amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48; and
- b . heavy chain CDR2 amino acid sequences: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ ID NO: 114.

In another aspect, the ET<sub>A</sub>R-1 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:



- a . light chain CDR3 amino acid sequences: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68; and
- b . heavy chain CDR3 amino acid sequences: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein (ET<sub>A</sub>R-2 antibody) comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR2 amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48; and
- b . heavy chain CDR2 amino acid sequences: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ ID NO: 114.

In one aspect, the ET<sub>A</sub>R-2 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR1 amino acid sequences: SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30; and
- b . heavy chain CDR1 amino acid sequences: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90.

In another aspect, the ET<sub>A</sub>R-2 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR3 amino acid sequences: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68; and
- b . heavy chain CDR3 amino acid sequences: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein (ET<sub>A</sub>R-3 antibody) comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR3 amino acid sequences: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68; and
- b . heavy chain CDR3 amino acid sequences: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

In one aspect, the ET<sub>A</sub>R-3 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a light chain CDR1 amino acid sequences: SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30; and
- b . heavy chain CDR1 amino acid sequences: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90.

In another aspect, the ET<sub>A</sub>R-3 antibody further comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . light chain CDR2 amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48; and
- b . heavy chain CDR2 amino acid sequences: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ ID NO: 114.

In one embodiment, the ET<sub>A</sub>R antibody provided herein comprises:

- a . a light chain CDR1 amino acid sequence independently selected from the list below:  
SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30;
- b . a light chain CDR2 amino acid sequence independently selected from the list below:  
SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48;
- c . a light chain CDR3 amino acid sequence independently selected from the list below:  
SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68;
- d . a heavy chain CDR1 amino acid sequence independently selected from the list below: SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, and SEQ ID NO: 90;
- e . a heavy chain CDR2 amino acid sequence independently selected from the list below: SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, and SEQ ID NO: 114; and

f . a heavy chain CDR3 amino acid sequence independently selected from the list below: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136.

In one embodiment, the ET<sub>AR</sub> antibody provided herein comprises a light chain CDR3 amino acid sequence independently selected from the list below: SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, and SEQ ID NO: 68. In another embodiment, the ET<sub>AR</sub> antibody provided herein comprises a heavy chain CDR3 amino acid sequence independently selected from the list below: SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, and SEQ ID NO: 136. In yet another embodiment, the ET<sub>AR</sub> antibody comprises a combination of light and heavy chain CDR3 amino acid sequences independently selected from the list below: SEQ ID NO: 50 *versus* SEQ ID NO: 116, SEQ ID NO: 62 *versus* SEQ ID NO: 128, SEQ ID NO: 62 *versus* SEQ ID NO: 130, SEQ ID NO: 64 *versus* SEQ ID NO: 132, SEQ ID NO: 66 *versus* SEQ ID NO: 134, and SEQ ID NO: 68 *versus* SEQ ID NO: 136.

In another embodiment, the ET<sub>AR</sub> antibody comprises 1 or 2 amino acid sequences, wherein each amino acid sequence is independently selected from the amino acid sequences listed below:

- a . amino acid sequences of light chain variable domains : SEQ ID NO: 138 (L1), SEQ ID NO: 140 (L2), SEQ ID NO: 142 (L3), SEQ ID NO: 144 (L4), SEQ ID NO: 146 (L5), SEQ ID NO: 148 (L6), SEQ ID NO: 150 (L7), SEQ ID NO: 152 (L8), SEQ ID NO: 154 (L9), SEQ ID NO: 156 (L10), SEQ ID NO: 158 (L11), SEQ ID NO: 160 (L12), SEQ ID NO: 162 (L13), SEQ ID NO: 164 (L14) and amino acid sequences at least 80%, 85%, 90% or 95% identical to one of the amino acid sequences listed above ; and
- b . amino acid sequences of heavy chain variable domains :SEQ ID NO: 166 (H1), SEQ ID NO: 168 (H2), SEQ ID NO: 170 (H3), SEQ ID NO: 172 (H4), SEQ ID NO: 174

(H5), SEQ ID NO: 176 (H6), SEQ ID NO: 178 (H7), SEQ ID NO: 180 (H8), SEQ ID NO: 182 (H9), SEQ ID NO: 184 (H10), SEQ ID NO: 186 (H11), SEQ ID NO: 188 (H12), SEQ ID NO: 190 (H13), and SEQ ID NO: 192 (H14), and amino acid sequences at least 80%, 85%, 90% or 95% identical to one of the amino acid sequences listed above.

In yet another embodiment, a polynucleotide coding sequence of the ET<sub>A</sub>R antibody provided herein comprises 1 or 2 polynucleotide sequences, wherein each polynucleotide sequence is independently selected from the polynucleotide sequences listed below:

- a . polynucleotide coding sequences of light chain variable domains: SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, SEQ ID NO: 163, and polynucleotide sequences at least 80%, 85%, 90% or 95% identical to one of the polynucleotide sequences listed above, and
- b . polynucleotide coding sequences of heavy chain variable domains: SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 181, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, and polynucleotide sequences at least 80%, 85%, 90% or 95% identical to one of the polynucleotide sequences listed above.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein comprises:

- a . an amino acid sequence of light chain variable domain independently selected from the list below: SEQ ID NO: 138 (L1), SEQ ID NO: 140 (L2), SEQ ID NO: 142 (L3), SEQ ID NO: 144 (L4), SEQ ID NO: 146 (L5), SEQ ID NO: 148 (L6), SEQ ID NO: 150 (L7), SEQ ID NO: 152 (L8), SEQ ID NO: 154 (L9), SEQ ID NO: 156 (L10), SEQ ID NO: 158 (L11), SEQ ID NO: 160 (L12), SEQ ID NO: 162 (L13), SEQ ID NO: 164 (L14), and amino acid sequences at least 80%, 85%, 90% or 95% identical to one of the amino acid sequences listed above, and
- b . an amino acid sequence of heavy chain variable domain independently selected from

the list below: SEQ ID NO: 166 (H1), SEQ ID NO: 168 (H2), SEQ ID NO: 170 (H3), SEQ ID NO: 172 (H4), SEQ ID NO: 174 (H5), SEQ ID NO: 176 (H6), SEQ ID NO: 178 (H7), SEQ ID NO: 180 (H8), SEQ ID NO: 182 (H9), SEQ ID NO: 184 (H10), SEQ ID NO: 186 (H11), SEQ ID NO: 188 (H12), SEQ ID NO: 190 (H13), SEQ ID NO: 192 (H14), and amino acid sequences at least 80%, 85%, 90% or 95% identical to one of the amino acid sequences listed above.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein comprises:

- a . an amino acid sequence of light chain variable domain independently selected from the list below: SEQ ID NO: 138 (L1), SEQ ID NO: 140 (L2), SEQ ID NO: 142 (L3), SEQ ID NO: 144 (L4), SEQ ID NO: 146 (L5), SEQ ID NO: 148 (L6), SEQ ID NO: 150 (L7), SEQ ID NO: 152 (L8), SEQ ID NO: 154 (L9), SEQ ID NO: 156 (L10), SEQ ID NO: 158 (L11), SEQ ID NO: 160 (L12), SEQ ID NO: 162 (L13), and SEQ ID NO: 164 (L14), and
- b . an amino acid sequence of heavy chain variable domain independently selected from the list below: SEQ ID NO: 166 (H1), SEQ ID NO: 168 (H2), SEQ ID NO: 170 (H3), SEQ ID NO: 172 (H4), SEQ ID NO: 174 (H5), SEQ ID NO: 176 (H6), SEQ ID NO: 178 (H7), SEQ ID NO: 180 (H8), SEQ ID NO: 182 (H9), SEQ ID NO: 184 (H10), SEQ ID NO: 186 (H11), SEQ ID NO: 188 (H12), SEQ ID NO: 190 (H13), and SEQ ID NO: 192 (H14).

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein comprises a combination of amino acid sequences of light and heavy chain variable domains independently selected from the list below: SEQ ID NO: 138 and SEQ ID NO: 166 (L1H1), SEQ ID NO: 140 and SEQ ID NO: 168 (L2H2), SEQ ID NO: 142 and SEQ ID NO: 170 (L3H3), SEQ ID NO: 144 and SEQ ID NO: 172 (L4H4), SEQ ID NO: 146 and SEQ ID NO: 174 (L5H5), SEQ ID NO: 148 and SEQ ID NO: 176 (L6H6), SEQ ID NO: 150 and SEQ ID NO: 178 (L7H7), SEQ ID NO: 152 and SEQ ID NO: 180 (L8H8), SEQ ID NO: 154 and SEQ ID NO: 182 (L9H9), SEQ ID NO: 156 and SEQ ID NO: 184 (L10H10), SEQ ID NO: 158 and SEQ ID NO: 186 (L11H11), SEQ ID NO: 160 and SEQ ID NO: 188 (L12H12), SEQ ID NO: 162 and SEQ ID NO: 190 (L13H13), and SEQ ID NO: 164 and SEQ ID NO: 192 (L14H14).

ET<sub>A</sub>R antibody provided herein can also be designated using the nomenclature "L<sub>x</sub>H<sub>y</sub>", wherein "x" corresponds to the sequence number of the light chain variable region and "y" corresponds to the sequence number of the heavy chain variable region. For example, L2H1 refers to an antibody with a light chain variable region comprising the amino acid sequence of SEQ ID NO: 140 (L2) and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 166 (H1).

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein comprises a light chain variable domain selected from L1-L14 or a heavy chain variable domain selected from H1-H14, and fragments, derivatives, muteins, or variants thereof.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein comprises a combination of light and heavy chain CDR3 amino acid sequences independently selected from the list below: SEQ ID NO: 138 and SEQ ID NO: 166, SEQ ID NO: 150 and SEQ ID NO: 178, SEQ ID NO: 152 and SEQ ID NO: 180, SEQ ID NO: 154 and SEQ ID NO: 182, SEQ ID NO: 156 and SEQ ID NO: 184, SEQ ID NO: 158 and SEQ ID NO: 186, SEQ ID NO: 160 and SEQ ID NO: 188, SEQ ID NO: 162 and SEQ ID NO: 190, and SEQ ID NO: 164 and SEQ ID NO: 192.

In one embodiment, the ET<sub>A</sub>R antibody provided herein comprises light chain variable domain amino acid sequence SEQ ID NO: 138 or heavy chain variable domain amino acid sequence SEQ ID NO: 166. In another embodiment, the ET<sub>A</sub>R antibody provided herein comprises the combination of light chain variable domain amino acid sequence SEQ ID NO: 138 and heavy chain variable domain amino acid sequence SEQ ID NO: 166.

In another embodiment, the ET<sub>A</sub>R antibody provided herein further comprises an amino acid sequence of a constant domain, wherein the amino acid sequence of the constant domain is independently selected from the amino acid sequences listed below:

- a .light chain constant domain amino acid sequences: SEQ ID NO: 194 and SEQ ID NO: 196; and
- b . heavy chain constant domain amino acid sequence: SEQ ID NO: 198.

In yet another embodiment, the ET<sub>A</sub>R antibody provided herein further comprises constant domain amino acid sequences, wherein each constant domain amino acid sequence

is independently selected from the combinations of the light chain and heavy chain constant domain amino acid sequences listed below:

- a . a combination of light chain constant domain amino acid sequence SEQ ID NO:194 and heavy chain constant domain amino acid sequence SEQ ID NO: 198; and
- b . a combination of light chain constant domain amino acid sequence SEQ ID NO:196 and heavy chain constant domain amino acid sequence SEQ ID NO: 198.

In one embodiment, the antibody provided herein comprises the amino acid sequences of the light and heavy chain CDRs and FRs (framework) illustrated above. In one embodiment, the antibody comprises a light chain CDR1 sequence illustrated above. In another embodiment, the antibody comprises a light chain CDR2 sequence illustrated above. In another embodiment, the antibody comprises a light chain CDR3 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain CDR1 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain CDR2 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain CDR3 sequence illustrated above. In another embodiment, the antibody comprises a light chain FR1 sequence illustrated above. In another embodiment, the antibody comprises a light chain FR2 sequence illustrated above. In another embodiment, the antibody comprises a light chain FR3 sequence illustrated above. In another embodiment, the antibody comprises a light chain FR4 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain FR1 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain FR2 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain FR3 sequence illustrated above. In another embodiment, the antibody comprises a heavy chain FR4 sequence illustrated above.

In one embodiment, a CDR3 sequence of the antibody differs from the combination of SEQ ID NO: 50 and SEQ ID NO: 116 of the light chain and heavy chain CDR3 sequences illustrated above by no more than 6, 5, 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In another embodiment, a light chain CDR3 sequence of the antibody differs from SEQ ID NO: 50 of the light chain CDR3 sequence illustrated above by no more than 6, 5, 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In another embodiment, a light chain CDR3 sequence of the antibody differs from SEQ ID NO: 50 of the light chain CDR3 sequence illustrated above by no more than 6, 5, 4, 3, 2 or 1 amino acid



addition(s), substitution(s), and/or deletion(s) and a heavy chain CDR3 sequence of the antibody differs from SEQ ID NO: 116 or SEQ ID NO: 118 of the heavy chain CDR3 sequence illustrated above by no more than 6, 5, 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In another embodiment, the antibody further comprises a combination of 1, 2, 3, 4, 5 or 6 of light and heavy chain CDR sequences illustrated above. In another embodiment, the antibody further comprises a combination of 1, 2, 3, 4, 5 or 6 of light and heavy chain CDR sequences illustrated above, wherein each sequence independently differs from a combination of SEQ ID NO: 50 and SEQ ID NO: 116 of light chain and heavy chain CDR3 sequences by 6, 5, 4, 3, 2 or 1 amino acid addition(s), substitution(s), and/or deletion(s). In another embodiment, the antibody comprises the CDRs of a light chain variable region and the CDRs of a heavy chain variable region illustrated above. In another embodiment, the antibody comprises a combination of 1, 2, 3, 4, 5, and/or 6 of light and heavy chain CDR sequences illustrated above.

In one embodiment, the antibody (such as an antibody or antibody fragment) comprises the amino acid sequence of light chain variable domain L1 illustrated above. In one embodiment, the sequence of the light chain variable domain differs from the sequence of light chain variable domain L1 by 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s), insertion(s), or substitution(s). In another embodiment, the light-chain variable domain comprises an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identical to the sequence of light chain variable domain L1. In another embodiment, the polynucleotide coding sequence of the light chain variable domain comprises a nucleotide coding sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identical to the polynucleotide coding sequence of L1. In another embodiment, the polynucleotide coding sequence of the light chain variable domain comprises a polynucleotide that hybridizes under moderately stringent conditions to the complement of the polynucleotide coding sequence of light chain variable domain L1. In another embodiment, the polynucleotide coding sequence of the light chain variable domain comprises a polynucleotide that hybridizes under stringent conditions to the complement of the polynucleotide coding sequence of light chain variable domain L1.

In one embodiment, the antibody (such as an antibody or antibody fragment)

comprises the amino acid sequence of heavy chain variable domain H1 illustrated above. In one embodiment, the sequence of the heavy chain variable domain differs from the sequence of heavy chain variable domain H1 by 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s), insertion(s), or substitution(s). In another embodiment, the heavy chain variable domain comprises an amino acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identical to the sequence of heavy chain variable domain H1. In another embodiment, a polynucleotide coding sequence of the heavy chain variable domain comprises a polynucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identical to the polynucleotide sequence of H1. In another embodiment, the polynucleotide coding sequence of the heavy chain variable domain comprises a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide coding sequence of the heavy chain variable domain H1. In another embodiment, the polynucleotide coding sequence of the heavy chain variable domain comprises a polynucleotide that hybridizes under stringent conditions to the complement of a polynucleotide coding sequence of the heavy chain variable domain H1.

In one embodiment, the antibody provided herein include the combination of L1H1、L2H2、L3H3、L4H4、L5H5、L6H6、L7H7、L8H8、L9H9、L10H10、L11H11、L12H12、L13H13 or L14H14; or an isotype thereof (for example, IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, IgE, or IgD) or a Fab or F(ab')<sub>2</sub> fragment thereof.

In one embodiment, the antibody provided herein includes an antibody comprising a combination of L1H1, or a converted isotype antibody thereof (for example, IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, IgE, or IgD) or a Fab or F(ab')<sub>2</sub> fragment thereof.

The antibody (*e.g.*, an antibody, antibody fragment, and antibody derivative) provided herein can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, *e.g.*, a murine kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon- gamma-, or mu-type heavy chain constant regions, *e.g.*, a murine alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

In one embodiment, the antibody provided herein further comprises a constant light chain  $\kappa$  or  $\lambda$  region or a fragment thereof. The light chain constant region sequence and its polynucleotide coding sequence are provided as follows:

Light chain constant region:

polynucleotide ( $\kappa$ ), (SEQ ID NO: 193); amino acid ( $\kappa$ ), (SEQ ID NO: 194)

polynucleotide ( $\lambda$ ), (SEQ ID NO: 195); amino acid ( $\lambda$ ), (SEQ ID NO: 196)

In another embodiment, the antibody provided herein further comprises a heavy chain constant region or a fragment thereof. The heavy chain constant region sequence and its polynucleotide coding sequence are provided as follows:

polynucleotide (IgG1), (SEQ ID NO: 197); amino acid (IgG1), (SEQ ID NO: 198)

In one embodiment, the ET<sub>AR</sub> antibody provided herein is selected from murine antibodies, humanized antibodies, chimeric antibodies, monoclonal antibodies, polyclonal antibodies, recombinant antibodies, antigen-binding antibody fragments, single-chain antibodies, double-chain antibodies, triple-chain antibodies, tetra-chain antibodies, Fab fragments, F(ab')<sub>x</sub> fragments, domain antibodies, IgD antibodies, IgE antibodies, IgM antibodies, IgG1 antibodies, IgG2 antibodies, IgG3 antibodies, and IgG4 antibodies. In another embodiment, the ET<sub>AR</sub> antibody provided herein is an ET<sub>AR</sub> monoclonal antibody. In yet another embodiment, the ET<sub>AR</sub> antibody provided herein is a mouse ET<sub>AR</sub> antibody. The ET<sub>AR</sub> antibody provided herein is a humanized ET<sub>AR</sub> antibody.

In one embodiment, the ET<sub>AR</sub> antibody provided herein is monoclonal antibody A-1 (comprising SEQ ID NO: 138 and SEQ ID NO: 166), A-7 (comprising SEQ ID NO: 150 and SEQ ID NO: 178), A-8 (comprising SEQ ID NO: 152 and SEQ ID NO: 180), A-9 (comprising SEQ ID NO: 154 and SEQ ID NO: 182), A-10 (comprising SEQ ID NO: 156 and SEQ ID NO: 184), A-11 (comprising SEQ ID NO: 158 and SEQ ID NO: 186), A-12 (comprising SEQ ID NO: 160 and SEQ ID NO: 188), A-13 (comprising SEQ ID NO: 162 and SEQ ID NO: 190), or A-14 (comprising SEQ ID NO: 164 and SEQ ID NO: 192).

#### Antibodies and Antibody Fragments

In one embodiment, the antibody provided herein is a full-length antibody (including polyclonal, monoclonal, chimeric, humanized or human antibody with full length heavy and/or light chains). In another embodiment, the antibody provided herein is an antibody fragment, for example, F(ab')<sub>2</sub>, Fab, Fab', Fv, Fc, or Fd fragment, and can be incorporated

into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, double-chain antibodies, triple-chain antibodies, tetra-chain antibodies, v-NAR and bis-scFv (*see e.g.*, Hollinger and Hudson, 2005, *Nature Biotechnology*, 23, 9, 1126-1136). In another embodiment, the antibody provided herein also includes antibody polypeptides such as those disclosed in U.S. Pat. No. 6703199, including fibronectin polypeptide monobodies. In another embodiment, the antibody provided herein also includes other antibody polypeptides disclosed in U.S. Patent Publication 2005/0238646, which are single-chain polypeptides.

In one embodiment, the variable regions of a gene expressing a monoclonal antibody of interest are amplified using nucleotide primers in a hybridoma. These primers can be synthesized by one of ordinary skill in the art, or can be purchased from commercially available sources (*see, e.g.*, Stratagene, La Jolla, Calif.), which sells primers for mouse and human variable regions including, among others, primers for V<sub>Ha</sub>, V<sub>Hb</sub>, V<sub>Hc</sub>, V<sub>Hd</sub>, C<sub>H1</sub>, V<sub>L</sub> and C<sub>L</sub> regions. These primers can be used to amplify heavy or light chain variable regions, which can then be inserted into vectors such as IMMUNOZAP<sup>TM</sup>H or IMMUNOZAP<sup>TM</sup>L (Stratagene), respectively. These vectors can then be introduced into *E. coli*, yeast, or mammalian-based systems for expression. Large amounts of a single-chain protein containing a fusion of the V<sub>H</sub> and V<sub>L</sub> regions can be produced using these methods (*see Bird et al.*, 1988, *Science* 242:423-426).

Once antibody-producing cells of the instant invention have been obtained using any above-described immunization and other techniques, the genes of the specific antibodies can be cloned by isolating and amplifying DNA or mRNA therefrom according to standard procedures described herein. The antibodies produced therefrom can be sequenced to identify CDRs, and the coding DNA of the CDRs can be manipulated as described above to generate other antibodies provided herein.

Antibodies provided herein preferably modulate endothelin signaling in the cell-based assay described herein and/or in the *in vivo* assay described herein and/or cross-block the binding of one of the antibodies described herein and/or are cross-blocked from binding ET<sub>A</sub>R by one of the antibodies described herein. Accordingly, such binding agents can be identified using the assays described herein.

In certain embodiments, antibodies are generated by first identifying antibodies that bind to cells overexpressing ET<sub>A</sub>R and/or neutralize in the cell-based and/or *in vivo* assays

described herein and/or cross-block the antibodies described herein and/or are cross-blocked from binding ET<sub>A</sub>R by one of the antibodies described herein.

It should be understood by one skilled in the art that certain proteins, such as antibodies, can undergo a variety of post-translational modifications. The types and extents of these modifications often depend on the host cell lines used to express the protein as well as the culture conditions. Such modifications can include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxyl-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, R. J., 1995, *Journal of Chromatography* 705:129-134).

An alternative method for production of a murine monoclonal antibody is to inject hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (*e.g.*, pristine-primed) to promote formation of ascites fluid containing the monoclonal antibody. Monoclonal antibodies can be isolated and purified by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (*see, e.g.*, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.*, "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, Vol. 10, pages 79-104 (The Humana Press, Inc. 1992)). A monoclonal antibody can be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the antibody (*e.g.*, heavy or light chain isotype, binding specificity, etc.). Examples of suitable ligands immobilized on a solid support include Protein A, Protein G, an anti-constant region (light chain or heavy chain) antibody, an anti-idiotypic antibody, and a TGF- $\beta$  binding protein, or a fragment or variant thereof.

Molecular evolution of the complementarity determining regions (CDRs) in the center of the antibody binding site also has been used to isolate antibodies with increased affinities, for example, antibodies having increased affinities for c-erbB-2, as described by Schier *et al.*, 1996, *J. Mol. Biol.* 263:551-567. Accordingly, such techniques are useful in preparing antibodies of human endothelin A receptor.

Antibodies against human endothelin A receptor can be used, for example, in assays to detect the presence of the endothelin A receptor, either *in vitro* or *in vivo*.

Antibodies can also be prepared by any of the conventional techniques. For example, they can be purified from cells that naturally express them (*e.g.*, an antibody can be purified from a hybridoma that produces it) or produced in recombinant expression systems using any technique known in the art. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988). This is discussed in the nucleic acid section below.

Antibodies can be prepared and screened for desired properties by any known techniques. Some techniques relate to the isolation of nucleic acids encoding polypeptide chains (or portions thereof) of related antibodies (*e.g.*, anti-ET<sub>A</sub>R antibodies) and manipulation of nucleic acid. Nucleic acids can be fused with another relevant nucleic acid or modified by recombinant DNA techniques (*e.g.*, induced mutations or other conventional techniques) to add, delete or replace one or more amino acid residues.

Where it is desired to improve the affinity of antibodies according to the invention containing one or more of the above-mentioned CDRs, such antibodies can be obtained by a number of affinity maturation protocols, including maintaining the CDRs (Yang *et al.*, 1995, *J. Mol. Biol.*, 254:392-403), chain shuffling (Marks *et al.*, 1992, *Bio/Technology*, 10:779-783), use of mutation strains of *E. coli*. (Low *et al.*, 1996, *J. Mol. Biol.*, 250:350-368), DNA shuffling (Patten *et al.*, 1997, *Curr. Opin. Biotechnol.*, 8:724-733), phage display (Thompson *et al.*, 1996, *J. Mol. Biol.*, 256:7-88) and additional PCR techniques (Crameri *et al.*, 1998, *Nature*, 391:288-291). All of these methods or affinity maturation are discussed in Vaughan *et al.*, 1998, *Nature Biotechnology*, 16:535-539).

In one embodiment, fragments of the ET<sub>A</sub>R antibody are provided herein. Such fragments can comprise entirely antibody-derived sequences or additional sequences. Examples of antigen binding fragments include Fab, F(ab')<sub>2</sub>, single chain antibodies, diabodies, tribodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde *et al.*, 2002, *Biochem. Soc. Trans.* 30:500-06.

Single chain antibodies can be formed by linking heavy and light chain variable domain (Fv region) fragments *via* an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusion DNA

encoding a peptide linker between DNAs encoding the two variable domain polypeptides ( $V_L$  and  $V_H$ ). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt *et al.*, 1997, *Prot. Eng.* 10:423; Kortt *et al.*, 2001, *Biomol. Eng.* 18:95-108). By combining different  $V_L$  and  $V_H$ -comprising polypeptides, multimeric scFvs that bind to different epitopes can be formed (Kriangkum *et al.*, 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Pat. No. 4946778; Bird, 1988, *Science* 242:423; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879; Ward *et al.*, 1989, *Nature* 334:544; de Graaf *et al.*, 2002, *Methods Mol. Biol.* 178:379-87. Single chain antibodies derived from antibodies provided herein including, but not limited to, scFvs comprising the variable domain combination L1H1, are encompassed by the present invention.

Antibodies derived from an antibody can also be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of a whole antibody according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a SS fragment termed F(ab')<sub>2</sub>. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. No. 4331647, Nisonoff *et al.*, 1960, *Arch. Biochem. Biophys.* 89:230; Porter, 1959, *Biochem. J.* 73:119; Edelman *et al.*, *Methods in Enzymology* 1:422 (Academic Press 1967); and by Andrews, S. M. and Titus, J. A. in *Current Protocols in Immunology* (Coligan J. E. , *et al.*, eds), John Wiley & Sons, New York (2003), pages 2.8.1-2.8.10 and 2.10A.1-2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light-heavy chain fragments (Fd), further cleaving of fragments, or other enzymatic, chemical, or genetic techniques can also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Another form of an antibody fragment is a peptide comprising one or more

complementarity determining regions (CDRs) of an antibody. CDRs can be obtained by constructing polynucleotides that encode the CDRs. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA or antibody-producing cells as a template (*see*, for example, Larrick *et al.*, 1991, Methods: A Companion to Methods in Enzymology 2:106; Courtenay-Luck, "(Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies: Production, Engineering and Clinical Application, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995); and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995). The antibody fragment further can comprise at least one variable region domain of an antibody described herein. Thus, for example, the V region domain can be monomeric and be a V<sub>H</sub> or V<sub>L</sub> domain, which can bind to ET<sub>A</sub>R with an affinity of  $1 \times 10^{-7}$  M or less as described below.

The variable region domain can be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

The variable region domain can be covalently attached at a C-terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a V<sub>H</sub> domain that is present in the variable region domain can be linked to an immunoglobulin C<sub>H1</sub> domain or a fragment thereof. Similarly, a V<sub>L</sub> domain can be linked to a C<sub>K</sub> domain or a fragment thereof. In this way, for example, the antibody can be a Fab fragment, wherein the antigen binding domain contains associated V<sub>H</sub> and V<sub>L</sub> domains covalently linked at their C-termini to a C<sub>H1</sub> and C<sub>K</sub> domain, respectively. The C<sub>H1</sub> domain can be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody C<sub>H2</sub> and C<sub>H3</sub> domains.

Derivatives and Variants of Antibodies



The nucleotide sequences of L1 and H1, encoding the corresponding amino acid sequences of A-1, can be altered, for example, by random mutagenesis or by site-directed mutagenesis (*e.g.*, oligonucleotide-directed site-specific mutagenesis) to create an altered polynucleotide comprising one or more particular nucleotide substitutions, deletions, or insertions as compared to the non-mutated polynucleotide. Examples of techniques for making such alterations are described in Walder *et al.*, 1986, *Gene* 42:133; Bauer *et al.*, 1985, *Gene* 37:73; Craik, 1985, *BioTechniques*, 3:12-19; Smith *et al.*, 1981, *Genetic Engineering: Principles and Methods*, Plenum Press; and U.S. Pat. Nos. 4518584 and 4737462. These and other methods can be used to make, for example, derivatives of anti-endothelin A receptor antibodies that have a desired property, for example, an increase in affinity, avidity, or specificity for an endothelin receptor or *in vivo* or *in vitro* stability, or reduced *in vivo* side-effects as compared to the underivatized antibody.

Other derivatives of anti-endothelin receptor antibodies within the scope of this invention include covalent or aggregative conjugates or anti-endothelin receptor antibodies, or fragments thereof, with other proteins or polypeptides, such as by expression or recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus or an anti-endothelin receptor antibody polypeptide. For example, the conjugated peptide can be a heterologous signal (or leader) polypeptide, *e.g.*, the yeast alpha-factor leader or a peptide such as an epitope tag. An antibody containing fusion proteins can comprise peptides added to facilitate purification or identification of antigen binding protein (*e.g.*, poly-His). An antibody also can be linked to the FLAG peptide as described in Hopp *et al.*, 1988, *Bio/Technology* 6:1204, and U.S. Pat. No. 5011912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of an expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, Mo.). In another embodiment, oligomers that contain one or more antibodies can be employed as endothelin receptor antagonists. Oligomers can be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antibodies are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers,

etc.

One embodiment is directed to oligomers comprising multiple antibodies joined *via* covalent or non-covalent interactions between peptide moieties fused to the antibodies. Such peptides can be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antibodies attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four antibodies. The antibodies of the oligomer can be in any form, such as any of the forms described above, *e.g.*, variants or fragments. Preferably, the oligomers comprise antibodies that have endothelin receptor binding activity.

In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi *et al.*, 1991, *PNAS USA* 88:10535; Byrn *et al.*, 1990, *Nature* 344:677; and Hollenbaugh *et al.*, 1992 "Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1-10.19.11. One embodiment provided herein is directed to a dimer comprising two fusion proteins created by fusing an endothelin receptor binding fragment of an anti-endothelin A receptor antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon inter-chain disulfide bonds form between the Fc moieties to yield the dimer.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby

incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Pat. No. 5457035 and in Baum *et al.*, 1994, *EMBO J.* 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors. In other embodiments, the variable portion of the heavy and/or light chains of an anti-endothelin receptor antibody can be substituted for the variable portion of an antibody heavy and/or light chain.

Alternatively, the oligomer is a fusion protein comprising multiple antibodies, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Pat. Nos. 4751180 and 4935233.

Another method for preparing oligomeric antibodies involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz *et al.*, 1988, *Science* 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe *et al.*, 1994, *FEBS Letters* 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow *et al.*, 1994, *Semin. Immunol.* 6:267-78. In one method, recombinant fusion proteins comprising an anti-endothelin receptor antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-endothelin receptor antibody fragments or derivatives that form are recovered from the culture supernatant.

In another embodiment, the antibody derivatives can comprise at least one of the CDRs disclosed herein. For example, one or more CDR can be incorporated into known antibody framework regions (IgG1, IgG2, etc.), or conjugated to a suitable vehicle to

enhance the half-life thereof. Suitable vehicles include, but are not limited to Fc, albumin, transferrin, and the like. These and other suitable vehicles are known in the art. Such conjugated CDR peptides can be in monomeric, dimeric, tetrameric, or other form. In one embodiment, one or more water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a binding agent. In an example, an antibody derivative comprises one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. *See, e.g.*, U.S. Pat. Nos. 4640835, 4496689, 4301144, 4670417, 4791192 and 4179337. In certain embodiments, a derivative comprises one or more of monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(*N*-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol, as well as mixtures of such polymers. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains. In certain embodiments, PEG can act to improve the therapeutic capacity for a binding agent, such as an antibody. Certain such methods are discussed, for example, in U.S. Pat. No. 6133426, which is hereby incorporated by reference for any purpose.

It will be appreciated that an antibody provided herein can have at least one amino acid substitution, providing that the antibody retains binding specificity. Therefore, modifications to the antibody structures are encompassed within the scope of the invention. These can include amino acid substitutions, which may be conservative or non-conservative, that do not destroy the human endothelin receptor binding capability of an antibody. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. A conservative amino acid substitution can also involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Non-conservative substitutions can involve the exchange of a member of one class of amino acids or amino acid mimetics for a member from another class with different physical properties (*e.g.*, size, polarity, hydrophobicity, charge).

Moreover, one skilled in the art may generate variants to be tested, which contain a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure. Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. A number of scientific publications have been devoted to the prediction of secondary structure. See Moulton, 1996, *Curr. Op. Biotech.* 7:422-427; Chou *et al.*, 1974,

*Biochemistry* 13:222-245; Chou *et al.*, 1974, *Biochemistry* 113:211-222; Chou *et al.*, 1978, *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45-148; Chou *et al.*, 1979, *Ann. Rev. Biochem.* 47:251-276 and Chou *et al.*, *Biophys. J.* 26:367-384. Moreover, computer programs are currently available to assist with predicting secondary structure. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within the structure of a polypeptide or protein. See Holm *et al.*, 1999, *Nucl. Acid. Res.* 27:244-247. It has been suggested (Brenner *et al.*, 1997, *Curr. Op. Struct. Biol.* 7:369-376) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

Additional methods of predicting secondary structure include “threading” (Jones, 1997, *Curr. Opin. Struct. Biol.* 7:377-87; Sippl *et al.*, 1996, *Structure* 4:15-19), “profile analysis” (Bowie *et al.*, 1991, *Science* 253:164-170; Gribskov *et al.*, 1990, *Meth. Enzym.* 183:146-159; Gribskov *et al.*, 1987, *Proc. Nat. Acad. Sci.* 84:4355-4358), and “evolutionary linkage” (see Holm, *supra* (1999), and Brenner, *supra* (1997)). In certain embodiments, variants of antibodies include glycosylation variants, wherein the number and/or type of glycosylation sites have been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, variants comprise a greater or lesser number of *N*-linked glycosylation sites than the native protein. Alternatively, elimination of such a sequence by substitutions removes an existing *N*-linked carbohydrate chain. Also provided is a rearrangement of *N*-linked carbohydrate chains, wherein one or more *N*-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new *N*-linked sites are created. Additional preferred antibody variants include cysteine variants, wherein one or more cysteine residues are deleted from or substituted for another amino acid (*e.g.*, serine) as compared to the parent amino acid sequence. Cysteine variants can be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to human endothelin receptor, or to increase or decrease the affinity of the antibodies to human endothelin receptor described herein.

According to certain embodiments, preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physiochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) can be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically cannot substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (Branden and Tooze, Eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, 1991, *Nature* 354:105, each of which is incorporated herein by reference.

In certain embodiments, antibodies of the invention can be chemically bonded with polymers, lipids, or other moieties.

The antigen binding agents can comprise at least one of the CDRs described herein incorporated into a biocompatible framework structure. In one embodiment, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a conformationally stable structural support, or framework, or scaffold, which is able to present one or more sequences of amino acids that bind to an antigen (*e.g.*, CDRs, a variable region, etc.) in a localized surface region. Such structures can be a naturally occurring polypeptide or polypeptide “fold” (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a

naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

Typically, the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostatin, cytochrome b, CP1 zinc finger, PST1, coiled coil, LACI-D1, Z domain and tendamistat domains can be used (*see, e.g., Nygren and Uhlen, 1997, Current Opinion in Structural Biology 7:463-469*).

Additionally, one skilled in the art will recognize that suitable binding agents include portions of these antibodies, such as one or more of heavy chain CDR1, CDR2, CDR3, light chain CDR1, CDR2 and CDR3 as specifically disclosed herein. At least one of the regions of heavy chain CDR1, CDR2, CDR3, CDR1, CDR2 and CDR3 can have at least one amino acid substitution, provided that the antibody retains the binding specificity of the non-substituted CDR. The non-CDR portion of the antibody can be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to human endothelin receptor and/or inhibits the activity of endothelin-1 signaling through the receptor. The non-CDR portion of the antibody can be a non-protein molecule in which the antibody exhibits a similar binding pattern to human endothelin receptor peptides in a competition binding assay as that exhibited by at least one of antibodies A1/A2, and/or neutralizes the activity of endothelin-1. The non-CDR portion of the antibody can be composed of amino acids, wherein the antibody is a recombinant binding protein or a synthetic peptide, and the recombinant binding protein cross-blocks the binding of an antibody disclosed herein to human ET<sub>A</sub>R and/or neutralizes endothelin-1 activity in vitro or in vivo. The non-CDR portion of the antibody can be composed of amino acids, wherein the antibody is a recombinant antibody, and the recombinant antibody exhibits a similar binding pattern to human ET<sub>A</sub>R peptides in a competition binding assay as exhibited by at least one of the antibodies A1/A2, and/or neutralizes endothelin-1 signaling.

#### Nucleic Acids

In one aspect, the present invention provides isolated nucleic acid molecules that encode the antibodies provided herein. The nucleic acids comprise, for example, polynucleotides that encode all or part of an antibody, for example, one or both chains of an



antibody of the invention, or a fragment, derivative, mutein, or variant thereof; polynucleotides sufficient for use as hybridization probes; PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide; anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (*e.g.*, peptide nucleic acids).

Nucleic acids encoding antibody polypeptides (*e.g.*, heavy or light chain, variable domain only, or full length) can be isolated from B-cells of mice that have been immunized with ET<sub>A</sub>R antigen. The nucleic acid can be isolated by conventional procedures such as polymerase chain reaction (PCR).

Nucleic acid sequences encoding the variable regions of the heavy and light chain variable regions are shown above. The skilled artisan will appreciate that, due to the degeneracy of the genetic code, each of the polypeptide sequences disclosed herein is encoded by a large number of other nucleic acid sequences. The present invention provides each degenerate nucleotide sequence encoding each antibody of the invention.

The invention further provides nucleic acids that hybridize to other nucleic acids (*e.g.*, nucleic acids comprising a nucleotide sequence of any of A-1/A-2) under particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. *See, e.g.*, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, for example, a moderately stringent hybridization condition uses a prewashing solution containing 5× sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of 55 °C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42 °C), and washing conditions of 60 °C, in 0.5×SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6×SSC at 45 °C, followed by one or more washes in 0.1×SSC, 0.2% SDS at 68 °C.

Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95, 98 or 99% identical to each other typically remain hybridized to each other. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and *Current Protocols in Molecular Biology*, 1995, Ausubel *et al.*, Eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (*e.g.*, an antibody) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues is changed using, for example, a random mutagenesis protocol. No matter how it is made, a mutant polypeptide can be expressed and screened for a desired property.

Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. In one embodiment, nucleotide sequences provided herein for L1 to L2 and H1 to H2, or fragments, variants, or derivatives thereof, are mutated such that they encode amino acid sequences provided herein for L1 to L2 and H1 to H2, comprising one or more deletions or substitutions of amino acid residues to result in sequences bearing two or more different amino acid residues. In another embodiment, the mutagenesis inserts an amino acid adjacent to one or more amino acid residues shown herein for L1 to L2 and H1 to H2 to result in sequences with two or more different amino acid residues. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively change the biological activity. (*e.g.*, binding to ET<sub>A</sub>R) of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative

changes include changing the antigen specificity of an antibody.

In another aspect, the present invention provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences of the invention. A nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide of the invention, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (*e.g.*, a ET<sub>AR</sub> binding portion) of a polypeptide of the invention.

Probes based on the sequence of a nucleic acid of the invention can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of the invention. The probe can comprise a label group, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

In another aspect, the vectors provided herein comprise a nucleic acid encoding a polypeptide of the invention or a portion thereof. Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors.

The recombinant expression vectors provided herein can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (*e.g.*, SV40 early gene enhancer, Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences, *see* Voss *et al.*, 1986, *Trends Biochem. Sci.* 11:287, Maniatis *et al.*, 1987, *Science* 236:1237, the disclosure of each of which is incorporated by reference herein in its entirety), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (*e.g.*, the metallothionin promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (*see Id.*). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be

transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

In another aspect, the present invention provides host cells into which a recombinant expression vector of the invention has been introduced. A host cell can be any prokaryotic cell or eukaryotic cell. Prokaryotic host cells include gram negative or gram positive organisms, for example, *E. coli* or *bacilli*. Higher eukaryotic cells include insect cells, yeast cells, and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (*see* Rasmussen *et al.*, 1998, *Cytotechnology* 28:31) or CHO strain DXB-11, which is deficient in DHFR (*see* Urlaub *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20). Additional CHO cell lines include CHO-K1 (ATCC#CCL-61), EM9 (ATCC# CRL-1861), and W20 (ATCC# CRL-1862). Additional host cells include the COS-7 line of monkey kidney cells (ATCC# CRL-1651) (*see* Gluzman *et al.*, 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL-163), AM-1/D cells (described in U.S. Pat. No. 6210924), HeLa cells, BHK (ATCC CRL-10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL-70) (*see* McMahan *et al.*, 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels *et al.* (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells can integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418,

hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

The transformed cells can be cultured under conditions that promote expression of a polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure is described in the Examples below. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti-endothelin receptor antibody polypeptides substantially free of contaminating endogenous materials.

#### Activity of Antibody

In one embodiment, the antibody provided herein specifically binds to an endothelin receptor, inhibits the signaling transduction, and demonstrates a therapeutic biological effect, for example, the attenuation of pulmonary arterial hypertension in an animal model. In another embodiment, a mouse or humanized antibody provided herein specifically binds to a human endothelin receptor. Such an antibody includes an antagonistic or neutralizing antibody that reduces or neutralizes endothelin signaling.

In one embodiment, the  $K_d$  of the antibody provided herein binding to a human endothelin receptor  $ET_{AR}$  is ranging approximately from 0.01 nM to 1000 nM, from 0.1 nM to 500 nM, from 0.5 nM to 200 nM, from 1 nM to 200 nM, or from 10 nM to 100 nM. In another embodiment, the  $K_d$  of the antibody provided herein binding to a human endothelin receptor  $ET_{AR}$  is approximately from 1 nM to 200 nM. In yet another embodiment, the  $K_d$  of the antibody provided herein binding to a human endothelin receptor  $ET_{AR}$  is approximately from 10 nM to 100 nM. In yet another embodiment, the  $K_d$  of the antibody provided herein binding to a human endothelin receptor  $ET_{AR}$  is approximately 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, or 100 nM.

In one embodiment, the  $IC_{50}$  of the antibody provided herein antagonizing endothelin signaling is ranging approximately from 0.01 nM to 500 nM, from 0.1 nM to 200 nM, from 0.5 nM to 200 nM, from 1 nM to 200 nM, or from 10 nM to 100 nM. In another embodiment, the  $IC_{50}$  of the antibody provided herein antagonizing endothelin signaling is approximately from 1 nM to 200 nM. In yet another embodiment, the  $IC_{50}$  of the antibody provided herein antagonizing endothelin signaling is approximately from 10 nM to 100 nM.

In yet another embodiment, the IC<sub>50</sub> of the antibody provided herein antagonizing endothelin signaling is approximately 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, or 100 nM.

In one embodiment, the antibody provided herein specifically binds to a human endothelin receptor ET<sub>A</sub>R with one or more following properties:

- a. having the same K<sub>d</sub> as a reference antibody in binding to a human endothelin receptor ET<sub>A</sub>R;
- b. having the same IC<sub>50</sub> as a reference antibody in antagonizing a human endothelin receptor ET<sub>A</sub>R activated by endothelin; and
- c. cross-competing binding with a reference antibody to a human endothelin receptor ET<sub>A</sub>R.

In one aspect, the reference antibody comprises a combination of light chain variable domain amino acid sequence SEQ ID NO: 138 and heavy chain variable domain amino acid sequence SEQ ID NO: 166. In another aspect, the reference antibody is monoclonal antibody A-1, A-2, A-7, A-9, or A-12.

As used herein, the term “substantially similar” means comparable to, or approximately 100%, 99%, 98%, 97%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, or 50% identical to the IC<sub>50</sub> or K<sub>b</sub> (or K<sub>d</sub>) of a reference antibody. In one embodiment, the reference antibody is, for example, an antibody comprising a heavy chain and light chain combination L1H1 or L2H2. In another embodiment, the reference antibody includes A-1.

In one embodiment, the ET<sub>A</sub>R antibody provided herein is able to bind to a human endothelin receptor specifically and lower pulmonary arterial hypertension in an animal model. In one embodiment, the pulmonary arterial hypertension is lowered by about 2% compared with an animal without treatment. In another embodiment, the pulmonary arterial hypertension is lowered by about 5% compared with an animal without treatment. In yet another embodiment, the pulmonary arterial pressure is lowered by about 10% compared to an animal without treatment. In yet another embodiment, the pulmonary arterial hypertension is lowered by about 15% compared to an animal without treatment. In yet another embodiment, the pulmonary arterial hypertension is lowered by about 20% compared to an animal without treatment. In yet another embodiment, the pulmonary arterial hypertension is lowered by about 25% compared to an animal without treatment. The

amount of reduction of pulmonary arterial hypertension is controlled by dosage. A therapeutically effective dosage is the dosage required to reduce pulmonary arterial hypertension into the normal range for an animal or human patient.

#### Pharmaceutical compositions

In one embodiment, the pharmaceutical composition provided herein comprises an ET<sub>A</sub>R antibody provided herein and one or more pharmaceutically acceptable carriers.

In one embodiment, a stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody is provided herein. The stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody is stable and efficacious with a longer half-life *in vivo*, and can be used to effectively treat pulmonary arterial hypertension and related diseases and cancer of a reproductive organ.

In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein comprises an ET<sub>A</sub>R antibody provided herein and a buffer. In one embodiment, the pH of the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein is ranging approximately from 4 to 11, from 5 to 7, or from 5 to 6. In another embodiment, the pH of the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein is approximately from 5 to 7. In yet another embodiment, the pH of the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein is approximately from 5 to 6. In yet another embodiment, the pH of the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein is approximately from 5.3 to 6.5. In still another embodiment, the pH of the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein is about 5.8.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody provided herein is approximately ranging from 10 to 500 mg/mL, from 10 to 250 mg/mL, from 10 to 200 mg/mL, or from 10 to 100 mg/mL. In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody provided herein is approximately 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, 50 mg/mL, 55 mg/mL, 60 mg/mL, 65 mg/mL, 70 mg/mL, 75 mg/mL, 80 mg/mL, 85 mg/mL, 90 mg/mL, 95 mg/mL, or 100 mg/mL. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody

provided herein is approximately 110 mg/mL, 120 mg/mL, 130 mg/mL, 140 mg/mL, 150 mg/mL, 160 mg/mL, 170 mg/mL, 180 mg/mL, 190 mg/mL, or 200 mg/mL. In still another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody provided herein is approximately 25 mg/mL, 50 mg/mL, 75 mg/mL, or 100 mg/mL.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the buffer described herein is ranging approximately from 1 mM to 200 mM, from 2 mM to 50 mM, or from 5 mM to 25 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the buffer described herein is approximately 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, or 50 mM. In still another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the buffer described herein is approximately 10 mM, 15 mM, 20 mM, 25 mM, or 30 mM.

In one embodiment, the buffer described herein comprises one or more selected from: citric acid, salts of citric acid, ascorbic acid, salts of ascorbic acid, gluconic acid, salts of gluconic acid, carbonic acid, salts of carbonic acid, tartaric acid, salts of tartaric acid, succinic acid, salts of succinic acid, acetic acid, salts of acetic acid, phthalic acid, salts of phthalic acid, phosphoric acid, salts of phosphoric acid, hydrochloric acid, Tris, thomethamine, and amino acids. In another embodiment, the buffer described herein is a salt of citric acid. In yet another embodiment, the buffer described herein is sodium citrate. In still another embodiment, the buffer described herein is histidine.

In another embodiment, the stable solution formulation of the ET<sub>A</sub>R antibody provided herein also comprises a surfactant.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the surfactant described herein is approximately ranging from 0.001 to 1 weight/volume percent, from 0.01 to 0.5 weight/volume percent, or from 0.01 to 0.1 weight/volume percent. In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the surfactant described herein is approximately from 0.01 to 0.1 weight/volume percent. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the surfactant described herein is approximately 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08,



0.09, or 0.1 weight/volume percent. In still another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the surfactant described herein is approximately 0.02, 0.03, 0.04, 0.05, or 0.06 weight/volume percent.

In one embodiment, the surfactant described herein is one or more selected from sorbitan fatty acid esters, glycerin fatty acid esters, polyglycerin fatty acid esters, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene glycerine fatty acid esters, polyethylene glycol fatty acid esters, polyoxyethylene alkyl ethers, polyoxyethylene polyoxypropylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene hydrogenated castor oils, polyoxyethylene beeswax derivatives, polyoxyethylene fatty acid amides, C10-C18 alkyl sulfates, polyoxyethylene C10-C16 alkyl ether sulfate with an average of 2 to 4 moles of the added oxirane groups, C1-C18 alkyl sulfosuccinate ester salts, natural surfactants, sphingophospholipids, and sucrose esters of C12-C18 fatty acids.

In another embodiment, the surfactant described herein is one or more selected from sorbitan fatty acid esters, *e.g.*, sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan trioleate; glycerin fatty acid esters, *e.g.*, glycerin monocaprylate, glycerin monomyristate, glycerin monostearate; polyglycerin fatty acid esters, *e.g.*, decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate; polyoxyethylene sorbitan fatty acid esters, *e.g.*, polyoxyethylene sorbitan monolaurate, wherein polyoxyethylene (20) sorbitan monolaurate is TWEEN-20 and polyoxyethylene sorbitan monopalmitate is TWEEN-40, polyoxyethylene sorbitan monooleate, wherein polyoxyethylene (80) sorbitan monooleate is TWEEN-80, polyoxyethylene sorbitan monostearate, wherein polyoxyethylene (60) sorbitan monostearate is TWEEN-60, polyoxyethylene sorbitan trioleate is TWEEN-85, and polyoxyethylene sorbitan tristearate is TWEEN-65; polyoxyethylene sorbitol fatty acid esters, *e.g.*, polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate; polyoxyethylene glycerine fatty acid esters, *e.g.*, polyoxyethylene glyceryl monostearate; polyethylene glycol fatty acid esters, *e.g.*, polyethylene glycol distearate; polyoxyethylene alkyl ethers, *e.g.*, polyoxyethylene lauryl ether; polyoxyethylene polyoxypropylene alkyl ethers, *e.g.*, polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether; polyoxyethylene alkylphenyl ethers, *e.g.*, polyoxyethylene

nonylphenyl ether; polyoxyethylene hydrogenated castor oils, *e.g.*, polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil; polyoxyethylene beeswax derivatives, *e.g.*, polyoxyethylene sorbitol beeswax; polyoxyethylene lanolin derivatives, *e.g.*, polyoxyethylene lanolin; and polyoxyethylene fatty acid amides, *e.g.*, polyoxyethylene stearic acid amide; C10-C18 alkyl sulfates, *e.g.*, sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate; polyoxyethylene C10-C16 alkyl ether sulfate with an average of 2 to 4 moles of ethylene oxide units added, *e.g.*, sodium polyoxyethylene lauryl sulfate; and C1-C18 alkyl sulfosuccinate ester salts, *e.g.*, sodium lauryl sulfosuccinate ester; and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipids, *e.g.*, sphingomyelin, and sucrose esters of C12-C18 fatty acids.

In one embodiment, the surfactant described herein is a polyoxyethylene sorbitan fatty acid ester, *e.g.*, TWEEN-20, TWEEN-40, TWEEN-60 and TWEEN-80. In another embodiment, the surfactant described herein is TWEEN-20 or TWEEN-80.

In another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody also comprises an amino acid protectant.

In one embodiment, in the stable solution formulation of ET<sub>AR</sub> antibody provided herein, the concentration of the amino acid protectant described herein is approximately ranging from 1 mM to 500 mM or from 10 mM to 200 mM. In another embodiment, in the stable solution formulation of the ET<sub>AR</sub> antibody provided herein, the concentration of the amino acid protectant described herein is approximately from 10 mM to 200 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>AR</sub> antibody provided herein, the concentration of the amino acid protectant described herein is approximately 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, or 200 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>AR</sub> antibody provided herein, the concentration of the amino acid protectant described herein is approximately 120 mM, 130 mM, 140 mM, 150 mM, or 160 mM.

In one embodiment, the amino acid protectant is one or more selected from histidine, arginine, glycine, and proline. In another embodiment, the amino acid protectant described herein is one or more selected from histidine, arginine, and glycine. In yet another embodiment, the amino acid protectant described herein is arginine or a salt thereof. In still another embodiment, the amino acid protectant described herein is arginine hydrochloride.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately from 10 to 200 mg/mL; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is approximately from 10 to 200 mM; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the formulation is approximately from 5 to 7.

In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, 75, or 100 mg/mL; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the amino acid protectant is approximately 140 mM; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5 to 6.

In yet another embodiment, the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein also comprises a polyol protectant.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the polyol described herein is approximately ranging from 0.1% to 50%, from 1% to 20%, from 1% to 15%, from 2% to 10%, or from 4% to 10%. In another embodiment, in the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the polyol described herein is approximately from 4 to 10 weight/volume percent. In yet another embodiment, in the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the polyol described herein is approximately 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 weight/volume percent.

In one embodiment, the polyol protectant described herein is sorbitol, mannitol, sucrose, or trehalose. In another embodiment, the polyol protectant described herein is sorbitol or mannitol.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R is approximately from 10 to 200 mg/mL; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is approximately from 1 to 20 weight/volume percent; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the

formulation is approximately from 5 to 7.

In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, 75 or 100 mg/mL; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the polyol protectant is approximately from 4 to 10 weight/volume percent; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5 to 6.

In another embodiment, the stable solution formulation of the ET<sub>A</sub>R antibody provided herein also comprises a metal chelator.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the metal chelator described herein is approximately ranging from 0.001 mM to 1 mM, from 0.005 mM to 0.5 mM, or from 0.01 mM to 0.2 mM. In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the metal chelator described herein is approximately from 0.01 mM to 0.2 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the metal chelator described herein is approximately 0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM, 0.06 mM, 0.07 mM, 0.08 mM, 0.09 mM, or 0.1 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the metal chelator described herein is approximately 0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM, 0.06 mM, 0.07 mM, 0.08 mM, 0.09 mM, or 0.1 mM. In still another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the metal chelator described herein is approximately 0.03 mM, 0.04 mM, 0.05 mM, 0.06 mM, or 0.07 mM.

In one embodiment, the metal chelator described herein is EDTA, DTPA, or EGTA. In another embodiment, the metal chelator described herein is EDTA.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately from 10 to 200 mg/mL; the concentration of the metal chelator is approximately from 0.01 to 0.2 mM; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is approximately from 10 to 200 mM; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the formulation is

approximately from 5 to 7.

In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, 75, or 100 mg/mL; the concentration of the metal chelator is approximately 0.05 mM; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the amino acid protectant is approximately 140 mM; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5 to 6.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately from 10 to 200 mg/mL; the concentration of the metal chelator is approximately from 0.01 mM to 0.2 mM; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is approximately from 1 to 20 weight/volume percent; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the formulation is approximately from 5 to 7.

In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, or 100 mg/mL; the concentration of the metal chelator is approximately 0.05 mM; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the polyol protectant is approximately from 4 to 10 weight/volume percent; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5 to 6.

In another embodiment, the stable solution formulation of the ET<sub>A</sub>R antibody provided herein also comprises an antioxidant.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the antioxidant described herein is approximately ranging from 0.1 mM to 50 mM, from 0.5 mM to 20 mM, or from 1 mM to 10 mM. In another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the antioxidant described herein is approximately from 1 to 10 mM. In yet another embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the antioxidant described herein is approximately 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, or 10 mM. In still another embodiment,

in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the antioxidant described herein is approximately 3 mM, 4 mM, 5 mM, 6 mM, or 7 mM.

In one embodiment, the antioxidant described herein is methionine, vitamin-C, thiosulfate, thiosulfate, or benzyl methionine. In another embodiment, the antioxidant described herein is methionine.

In one embodiment, in the stable solution formulation of the ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately from 10 to 200 mg/mL; the concentration of the antioxidant is approximately from 1 to 10 mM; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is approximately from 10 to 200 mM; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the formulation is approximately from 5 to 7.

In another embodiment, in the stable solution formulation of ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, 75, or 100 mg/mL; the concentration of the antioxidant is approximately 5 mM; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the amino acid protectant is approximately 140 mM; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5 to 6.

In one embodiment, the stable solution formulation of ET<sub>A</sub>R antibody provided herein, in the concentration of the ET<sub>A</sub>R antibody is approximately from 10 to 200 mg/mL; the concentration of the antioxidant is approximately from 1 mM to 10 mM; the concentration of the surfactant is approximately from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is approximately from 1 to 20 weight/volume percent; and the concentration of the buffer is approximately from 1 to 50 mM; wherein the pH of the formulation is approximately from 5 to 7.

In another embodiment, in the stable solution formulation of ET<sub>A</sub>R antibody provided herein, the concentration of the ET<sub>A</sub>R antibody is approximately 25, 50, 75, or 100 mg/mL; the concentration of the antioxidant is approximately 5 mM; the concentration of the surfactant is approximately 0.04 weight/volume percent; the concentration of the polyol protectant is approximately from 4 to 10 weight/volume percent; and the concentration of the buffer is approximately 20 mM; wherein the pH of the formulation is approximately from 5

to 6.

In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein comprises:

- an ET<sub>AR</sub> monoclonal antibody, at 10-150 mg/mL;
- a metal chelator at 0.1 mM to 1 mM;
- a surfactant at 0.01% to 0.1%;
- a polyol protectant at 1% to 50% or an amino acid protectant at 10 to 200 mM; and
- a buffering system, providing a pH of 5.0-7.0.

In another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein comprises:

- an ET<sub>AR</sub> monoclonal antibody at 10 to 150 mg/mL;
- a metal chelator at 0.02 mM to 0.2 mM;
- a surfactant at 0.01% to 0.1%;
- a polyol protectant at 1% to 10% or an amino acid protectant 50 to 200 mM; and
- a buffering system, providing a pH of 5.3-6.5.

In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein comprises:

- an ET<sub>AR</sub> monoclonal antibody at 10 to 150 mg/mL;
- a metal chelator at 0-1 mg/mL;
- a surfactant at 0-0.1%;
- a polyol protectant at 0-50% or an amino acid protectant at 0-200 mM; and
- a buffering system, providing a pH of 5.0-7.0.

In another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein comprises:

- an ET<sub>AR</sub> monoclonal antibody at 10-150 mg/mL;
- EDTA at 0-0.1 mg/mL
- a surfactant at 0-0.1%;
- a polyol protectant at 0-10% or an amino acid protectant at 50-200 mM; and
- a buffering system, providing a pH of 5.3-6.5.

In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein is an aqueous solution. In another embodiment, the stable

formulation of the pharmaceutical ET<sub>AR</sub> antibody provided herein is a sterile solution.

In one embodiment, the stability of the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein is determined by the extent of aggregation of the ET<sub>AR</sub> antibody. In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at about 40 °C and about 75% humidity for 1, 2, 3, 6, 12, or 24 months, contains no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1% of an aggregated ET<sub>AR</sub> antibody. In another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at room temperature and about 65% humidity for 3, 6, 12, or 24 months, contains no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1% of an aggregated ET<sub>AR</sub> antibody. In yet another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at 2-8 °C for 6, 12, 18, 24, 36 or 48 months, contains no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1% of an aggregated ET<sub>AR</sub> antibody.

In another embodiment, the stability of the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein is determined by the extent of degradation of the ET<sub>AR</sub> antibody. In one embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at about 40 °C and about 75% humidity for 1, 2, 3, 6, 12 or 24 months, has a degree of degradation of the ET<sub>AR</sub> antibody of no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1%. In another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at room temperature and about 65% humidity for 3, 6, 12, or 24 months, has a degree of degradation of the ET<sub>AR</sub> antibody of no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1%. In yet another embodiment, the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein, after stored at 2-8 °C for 6, 12, 18, 24, 36 or 48 months, has a degree of degradation of the ET<sub>AR</sub> antibody of no more than 20%, 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1%.

In one embodiment, the aggregation of the ET<sub>AR</sub> antibody and the loss of a monomeric ET<sub>AR</sub> antibody are determined by SEC-HPLC.

In another embodiment, the stability of the stable pharmaceutical solution formulation of the ET<sub>AR</sub> antibody provided herein is determined by the change in the biological activity of the ET<sub>AR</sub> antibody. In one embodiment, the ET<sub>AR</sub> antibody in the stable pharmaceutical



solution formulation of the ET<sub>A</sub>R antibody provided herein, after stored at about 40 °C and about 75% humidity for 1, 2, 3, 6, 12 or 24 months, has a biological activity of no less than 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.9% of its original biological activity. In another embodiment, the ET<sub>A</sub>R antibody in the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein, after stored under room temperature and about 65% humidity for 3, 6, 12, or 24 months, has a biological activity of no less than 50%, 60%, 70%, 80%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% of its original biological activity. In yet another embodiment, the ET<sub>A</sub>R antibody in the stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody provided herein, after stored under temperature of 2-8 °C for 6, 12, 18, 24, 36 or 48 months, has a biological activity of no less than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% of its original biological activity.

In one embodiment, the change in the biological activity of the ET<sub>A</sub>R antibody is determined by a calcium flux detection method to determine the ability of an ET<sub>A</sub>R antibody to inhibit an ET<sub>A</sub>R *in vitro*.

#### Methods of treatment

In one embodiment, provided herein is a method of lowering hypertension (for example, PAH) in a subject, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition provided herein, for example, a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody provided herein.

In another embodiment, provided herein is a method of treating PAH in a subject, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition provided herein, for example, a stable pharmaceutical solution formulation of an ET<sub>A</sub>R antibody provided herein.

As used herein, the term “subject” refers to a mammal, including humans, and is used interchangeably with the term “patient.” The term “treatment” encompasses alleviation or prevention of at least one symptom or other aspect of a disorder, or reduction of disease severity, and the like. An antibody provided herein needs not to provide a complete cure, or to eradicate every symptom or manifestation of a disease, to be an effective therapeutic agent. As is recognized in the pertinent field, therapeutic agents can reduce the severity of a given disease state, but need not to abolish every manifestation of the disease to be effective.

Similarly, a prophylactic agent needs not to prevent the onset of a condition completely in order to be effective. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient. One embodiment of the invention is directed to a method comprising administering to a patient an antibody in an amount and for a time sufficient to induce a sustained improvement over baseline of an indicator that reflects the severity of a particular disorder.

A pharmaceutical composition can be administered by any suitable technique, including, but not limited to, parenterally, topically, or by inhalation. If injected, the pharmaceutical composition can be administered, for example, via an intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous route, by bolus injection or continuous infusion. It is considered, for example, localized administration at the disease or injury site, such as transdermal administration and sustained release of an implant. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebulizer, inhalation of an antibody in aerosol form, and the like. Other alternatives include oral preparations, including pills, syrups, or lozenges.

Advantageously, the antibodies provided herein, are administered in a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. The composition additionally comprises one or more physiologically active agents as described below. In many particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to one or more antibodies (*e.g.*, murine antibodies or humanized antibodies) provided herein.

In one embodiment, the pharmaceutical composition comprises a murine antibody or humanized antibody of the invention together with one or more substances selected from the group consisting of a buffer suitable for the antibody at a suitable pH, an antioxidant such as ascorbic acid, a low molecular weight polypeptide (such as those having fewer than 10 amino acids), a protein, an amino acid, a carbohydrate such as dextrin, a chelating agent such as EDTA, glutathione, a stabilizer, and an excipient. In accordance with appropriate industry standards, preservatives can also be added. The composition can be formulated as a lyophilizate using appropriate excipient solutions as diluents. Suitable components are

nontoxic to recipients at the dosages and concentrations employed. Further examples of components that can be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16th Ed. (1980) and 20th Ed. (2000). Mack Publishing Company kits for use by medical practitioners are provided, including one or more antibodies of the invention and a label or other instructions for use in treating any of the conditions discussed herein. In one embodiment, the kit includes a sterile preparation of one or more human antibodies, which can be in the form of a composition as disclosed above, and can be in one or more vials.

Dosages and the frequency of administration can vary according to such factors as the route of administration, the particular antibodies employed, the nature and severity of the disease to be treated, whether the condition is acute or chronic, and the size and general condition of the subject. Appropriate dosages can be determined by procedures known in the pertinent art, e.g. in clinical trials that can involve dose escalation studies.

An antibody provided here can be administered, for example, once or more than once, e.g., at regular intervals over a period of time. In particular embodiments, a murine antibody or humanized antibody is administered over a period of at least once a month or more, e.g., for one, two, or three months or even indefinitely. For treating chronic conditions, long-term treatment is generally most effective. However, for treating acute conditions, administration for shorter periods, e.g., from one to six weeks, can be sufficient. In general, the humanized antibody is administered until the patient manifests a medically relevant degree of improvement over baseline for the chosen indicator or indicators.

One example of therapeutic regimens provided herein comprise subcutaneous injection of an antibody once a week, at an appropriate dosage, to treat a condition in which pulmonary arterial pressure levels play a role. Weekly or monthly administration of antibody would be continued until a desired result is achieved, e.g., the subject's symptoms subside. Treatment can resume as needed, or, alternatively, maintenance doses can be administered.

A subject's levels of pulmonary arterial pressure can be monitored before, during and/or after treatment with an antibody such as a humanized antibody, to detect changes, if any, in their levels. For some disorders, the incidence of elevated pulmonary arterial pressure can vary according to such factors as the stage of the disease. Known techniques

can be employed for measuring pulmonary arterial pressure levels.

Particular embodiments of methods and compositions of the invention involve the use of an antibody and one or more ET<sub>A</sub>R antagonists for example, two or more antibodies of the invention, or an antibody of the disclosure and one or more other ET<sub>A</sub>R antagonists. In further embodiments, an antibody is administered alone or in combination with other agents useful for treating the condition with which the patient is afflicted. Examples of such agents include both proteinaceous and non-proteinaceous drugs. When multiple therapeutics are co-administered, dosages can be adjusted accordingly, as is recognized in the pertinent art. “Co-administration” and combination therapy are not limited to simultaneous administration, but also include treatment regimens in which an antibody is administered at least once during a course of treatment that involves administering at least one other therapeutic agent to the patient.

In another aspect, the method of preparing a medicament for treating pulmonary arterial hypertension and related disorders comprises a mixture of the antibody provided herein and pharmaceutically acceptable excipients. The preparation method of the medicament was as described above.

A composition, kit, and method related to an antibody specifically binding to a human endothelin receptor are further provided herein. Nucleic acid molecules and derivatives and fragments thereof comprising a part of or a full polynucleotide encoding a polypeptide interacting with an ET<sub>A</sub>R, for example, nucleic acids encoding all or part of an endothelin receptor antibody, an antibody fragment or an antibody derivative, are also provided. A vector and plasmid comprising nucleic acids and cells and cell line comprising nucleic acids and/or a vector and plasmid are further provided herein. Methods provided herein include, for example, methods for preparation, identification or separation of an antibody interacting with a human ET<sub>A</sub>R, for example, a method of an ET<sub>A</sub>R antibody, a method for determining whether an antibody binds to ET<sub>A</sub>R, and a method for administering an antibody binding to an ET<sub>A</sub>R to an animal model.

The technical solutions described herein will be further understood by the following examples.

## **EXAMPLES**

If not specified, the starting materials and equipment described herein are

commercially available or commonly used in the art. The methods in the following examples, unless otherwise specified, are all conventional methods in the art.

#### 1. Construction of a stable antigen cell line for immunization

CHO-DHFR- cells were seeded into a 6-well plate. After 24 h culture, the cells were transfected with a pIRES plasmid (Clontech, commercial) modified to carry hET<sub>A</sub>R gene (*see* SEQ ID NO: 1 for the nucleotide sequence, and SEQ ID NO: 2 for the amino acid sequence). The transfection was carried out by following the transfection conditions recommended by Invitrogen for Lipofectamine 2000. Forty-eight hours after transfection, the medium was replaced with a complete medium containing 10 nM MTX (methotrexate). The medium was changed every 3 days for about two weeks until stable clones appeared. The dispersed cell colonies were detached from the plate and collected. After cells grew to about 50% confluence, the concentration of MTX was gradually increased for pressure selection up to 10  $\mu$ M. The constructed stable cell lines were analyzed by FACS using a polyclonal antibody (Abcam) against hET<sub>A</sub>R to identify cell clones after pressure selection. A large amount of hET<sub>A</sub>R expression were detected on the selected CHO-DHFR-hET<sub>A</sub>R cell membranes. Finally through subcloning, six high-ET<sub>A</sub>R expression and stable cell lines were identified and obtained.

#### 2. Preparation of antibodies

An emulsion of the CHO-DHFR-hET<sub>A</sub>R whole cells and Freund's adjuvant was injected subcutaneously into BALB/c mice (6-8 weeks) at  $2 \times 10^6$  cells/mouse. After 2 weeks, the mice were boosted with incomplete Freund's adjuvant emulsified immunogen and then boosted once every week. After immunization for 6 times in total, blood samples were collected from the clipped tail ends and centrifuged to collect the serum. The serum was analyzed for serum titers by FACS. After the acceptable antibody titers were achieved, the mice were sacrificed and their spleen cells were harvested under aseptic conditions. SP2/0 cells were collected at the logarithmic phase of growth with 3 min centrifugation at 2,000 rpm. The cell pellets were resuspended with serum-free culture medium, then centrifuged, resuspended for a second time and counted. Spleen cells and SP2/0 cells were mixed at ratio of SP2/0 cells : spleen cells  $\geq 1:1$ , followed by 3 rounds of washing-centrifugation. After the pellets from the last centrifugation were detached, 1 mL of pre-warmed PEG-1350 was added dropwise (finished in 30 s), after pipette-mixing for 1 min, 30 mL of the

pre-warmed serum-free medium (Invitrogen) was added slowly to terminate the PEG fusion. After 5 min centrifugation at 1,500 rpm, the cell pellets were resuspended in the fusion culture medium. Spleen cells (20,000) and feeder layer cells (5,000) in 100  $\mu$ L were plated into each well of 96-well plates. Fused hybridoma cells and feeder layer cells were co-cultured in 96-well plates with HAT (sarcine, amethopterin and thymidine) selection to get rid of the non-fused cells. After 10 days, the supernatants of the hybridoma cells in the culture plates were collected for ELISA analysis.

### 3. ELISA screening of whole cells

CHO-DHFR-hET<sub>A</sub>R cells over-expressing hET<sub>A</sub>R and CHO-DHFR- cells not expressing hET<sub>A</sub>R were separately transferred into a 96-well plate and allowed to grow to 90% confluent. The supernatant of the culture medium was removed and attached cells were washed twice with PBS, then 100  $\mu$ L, 100% methanol was added to fix the cells for 10 min at 4 °C. Then 100  $\mu$ L freshly made 0.6% H<sub>2</sub>O<sub>2</sub>-PBS was added, and after incubation at room temperature for 20 min, the cells were washed twice with PBS. After blocked with PBS-1% BSA solution, the hybridoma supernatant was added and incubated for 90 min at 4 °C. After several washes, 100  $\mu$ L of the secondary antibody GxM-HRP-Fc (Sigma-Aldrich) was added into each well and incubated at 37 °C for 0.5 h. After five washings, 100  $\mu$ L of TMB chromogenic substrate was added into each well and incubated at 37 °C for 15 min, and then 2M H<sub>2</sub>SO<sub>4</sub> was added to terminate, read for OD<sub>450</sub> values. The positive control was the mouse serum after immunization; the negative control was the cell culture supernatant. As shown in Figure 1, after initial analysis by ELISA, several hybridoma clones secreting anti-hET<sub>A</sub>R antibodies were selected, and the stable secretory cell lines against hET<sub>A</sub>R were obtained after cell cloning. Lastly, antibody supernatant secreted by hybridoma was verified by FACS analysis.

### 4. Cloning and subcloning of antibody genes

Hybridoma cells secreting antibodies were collected. Hybridoma mRNA was extracted according to the manufacturer protocol of QIAGEN mRNA extraction kit. Then the extracted mRNA was transcribed reversely into cDNA. The reverse transcription primers were specific primers for the light and heavy chain constant regions of a mouse, with the heavy chain reverse transcription primer being (5'-TTTGGRGGGAAGATGAAGAC-3') (SEQ ID NO: 199), the light chain reverse transcription primers being

(5'-TTAACACTCTCCCCTGTTGAA-3') (SEQ ID NO: 200) and (5'-TTAACACTCATTCCTGTTGAA-3') (SEQ ID NO: 201). RT-PCR reaction conditions were as following: 25 °C for 5 min, 50 °C for 60 min, and 70 °C for 15 min. Reversely transcribed cDNA was diluted with 0.1 mM TE to 500 µL, added into the ultrafiltration centrifuge tube (Amicon Ultra-0.5) and centrifuged at 2,000 g for 10 min. The filtrate was removed, 500 µL of 0.1 mM TE were added and centrifuged at 2,000 g for 10 min. The filtrate was removed and the preparation tube was placed in inversion to the new centrifugal tube, and centrifuged at 2,000 g for 10 min to obtain the purified cDNA. Purified cDNA (10 µL) was taken as a template, followed by addition of 4 µL 5x tailing buffer (Promega), 4 µL dATP (1 mM) and 10 U terminal transferase (Promega), mixing uniformly, and incubation at 37 °C for 5 min and then at 65 °C for 5 min. The PolyA tail cDNA was used as a template and PCR was performed to amplify light and heavy chain variable region genes of antibodies. Upstream primers were all oligodT, with heavy chain downstream primers being (5'-TGGACAGGGATCCAGAGTTCC-3') (SEQ ID NO: 202) and (5'-TGGACAGGGCTCCATAGTTCC-3') (SEQ ID NO: 203), and light chain downstream primer being (5'-ACTCGTCCTTGGTCAACGTG-3') (SEQ ID NO: 204). The PCR reaction conditions were: 95 °C for 5 min; 95 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min, 40 cycles; and 72 °C for 7 min. The PCR products were connected to the PMD 18-T vector (Takara Bio) for sequencing. The sequences of the antibody clones were listed in Table 2.

PCR primers were designed based on the DNA sequences of the antibodies, thus the complete light chain, heavy chain signal peptides and variable domains and mouse IgG1 constant region were ligated into expression vector pTM5.

## 5. Antibody humanization and optimization

First of all, the sequences of light and heavy chain variable regions of the screened mouse antibodies were aligned with the homologous antibodies, using NCBI online antibody variable region sequence alignment tool (Ig Blast) to search the germline gene sequences of a humanized antibody (Ig Germline Gene sequence) homologous to the selected antibodies variable region sequence for humanization, and the humanized gene sequence with highest homology except CDR sequences was used as a template for CDR grafting to obtain humanized antibody variable region sequences and to synthesize humanized antibody light and heavy chain genes through a CRO. According to the sequences, PCR primers were

designed and appropriate restriction enzyme sites were introduced at the 5' ends and 3' ends. By PCR, the humanized antibody variable regions were amplified and then combined with the human IgG2 or IgG4 constant region sequence to obtain whole recombinant humanized antibody sequences. The expression of the recombinant antibodies was achieved according to step 7, and their affinities to ET<sub>A</sub>R was analyzed by FACS as described in step 9. The best humanized antibody candidate retaining affinity to ET<sub>A</sub>R was selected from the group, and its variable region sequence was further improved by site-specific mutagenesis for improved affinity to ET<sub>A</sub>R.

#### 6. Subcloning of genes of a humanized anti-hET<sub>A</sub>R antibody

The heavy and light chain variable region gene sequences of an optimized humanized antibody were synthesized by Genscript Biotechnology CO., LTD by introducing two restriction sites of NheI at the 5'-end and SalI at the 3'-end. The whole heavy chain variable region was ligated with a heavy chain constant region in an expression vector of pTM5. Similarly, by introducing NheI at the 5'-end and BsiwI at the 3'-end, the light chain variable region was ligated with a light chain constant region in the expression vector of pTM5.

#### 7. Transient expression of anti-ET<sub>A</sub>R antibodies

A suspension of an HEK293 or CHO expressing cell line ( $5 \times 10^5$ /mL) was inoculated to a shaker flask. After 24 h rotation at 37 °C, the cell density reached  $1 \times 10^6$ /mL and were ready for transfection. Polyethylenimine (PEI) was used as a transfection reagent with an optimal mixing ratio of 3:1 for PEI to DNA (DNA amount,  $0.5 \mu\text{g/L} \times 10^6$  cells; the ratio of the antibody light chain DNA and antibody heavy chain DNA, 3:2). A mixture of both was added into the cell culture after 15 min incubation. The cells after treated with the PEI/DNA mixture were rotated for more than 24 h at 37°C and 5% CO<sub>2</sub>. Then 0.5% of tryptone was added into the cell culture as a supplement required by expression, and after the completion of expression (more than 96 h), the cell supernatant was collected for the antibody purification and separation.

#### 8. Purification and preparation of antibody

Cells and cellular debris were removed from the culture after centrifugation (8000 rpm, 15 min), and the supernatant was filtered through 0.45  $\mu\text{m}$  filter for purification. The purification process was done through chromatography. First, the supernatant was passed



through a G protein coupled affinity chromatography column, and antibodies bound to the G proteins remained in the column. The antibodies were eluted from the chromatography column using an eluent with pH of 3.0 or less. The low pH eluent was neutralized immediately with 1M Tris-HCl to keep the antibodies from denaturation and loss of activity. The antibody solution was then dialyzed over 16 h into a PBS buffer.

#### 9. FACS analysis of a functional antibody

PBS containing 10 mM EDTA was used to detach and collect  $10^5$  CHO-DHFR-hET<sub>A</sub>R cells into a 1.5 mL EP tube. The supernatant was removed after centrifugation and the negative control sample was resuspended with a loading buffer (PBS, 2% FBS). For the positive control, 200  $\mu$ L antibody supernatant was added to resuspension cells and incubation at room temperature; the cells were then centrifuged at 1500 rpm to remove the supernatant, washed with a FACS loading buffer and centrifuged again. The cells were resuspended with addition (200  $\mu$ L/well) of a FITC labeled goat anti-mouse fluorescent antibody at 1:50 dilution (BD Pharmingen) and incubated at room temperature for 30 min in the dark. Supernatant was removed after centrifugation, cells were washed with FACS loading buffer, centrifuged again and resuspended with the loading buffer for analysis. The recombinant antibody supernatant and CHO-DHFR-hET<sub>A</sub>R cells had specific binding. Gray peak and dotted line peak were negative controls; the solid line peak, corresponding to the antibody supernatant, moved to the right significantly.

#### 10. Calcium influx assay for a functional antibody

CHO-DHFR cells co-expressing hET<sub>A</sub>R-Aequorin were seeded into a 96-well cell culture plate with 25000 cells per well and cultured at 37 °C overnight. The next day the culture supernatant was removed. Coelenterazine (50  $\mu$ L) (Promega) was added in the dark and incubated at 37 °C for 2 h, and then 50  $\mu$ L of a hybridoma supernatant or a purified antibody were added and incubated at 37 °C for 30 min. After the incubation, 50  $\mu$ L endothelin 1 was added and the changes of calcium influx within 40 s were recorded by a SpectraMax L microplate reader (Molecular Devices). Different hybridoma supernatants inhibited the calcium influx mediated through hET<sub>A</sub>R differently, and A-1 antibody significantly inhibited the calcium influx mediated through hET<sub>A</sub>R. The recombinant anti-hET<sub>A</sub>R functional antibody significantly inhibited calcium influx mediated through hET<sub>A</sub>R, increasing with an increase in the antibody concentration.

11. Establishment of hypoxia-induced PAH cynomolgus model to study the *in vivo* activity of an antibody

The acute hypoxia-induced pulmonary arterial hypertension (PAH) model of cynomolgus was codeveloped with Crown Bioscience Inc. (Taicang), and the efficacy of A-1 antibody as a single intravenous injection was evaluated in this PAH model. All animals were fasted overnight and weighed, and then received a single intravenous injection of 10 mg/kg of A-1 antibody. Three hours later, the animals were anesthetized. The tricuspid regurgitation velocity by Doppler color echocardiography along with heart rate and oxygen saturation were monitored simultaneously. The baseline was obtained and the induction of 12% hypoxia was followed and at the same time the tricuspid regurgitation velocity was measured; Analysis was made to determine if the antibody would improve hypoxia-induced pulmonary arterial pressure under 12% hypoxia. After 48 h of administration, the tests were performed again. The animals were anesthetized, the tricuspid regurgitation velocity by Doppler color echocardiography along with heart rate and oxygen saturation were monitored simultaneously. The baseline was obtained and the induction of 12% hypoxia was followed and at the same time the tricuspid regurgitation velocity was measured. Analysis was made to determine if the antibody would still improve hypoxia-induced pulmonary arterial pressure. If the efficacy maintained after 48 h, 96 h later, hypoxia induction experiment was performed again. The area under the curve of pulmonary artery systolic pressure versus time was calculated, and by comparing the area under the curve, it was found that A-1 maintained the efficacy of reducing pulmonary artery pressure within 96 h.

12. Stable pharmaceutical solution formulations of an ET<sub>A</sub>R antibody:

The followings are examples of stable pharmaceutical solution formulations of an ET<sub>A</sub>R antibody:

Example 1 :

Ingredients	Concentration
ET <sub>A</sub> R antibody A-1	25 mg/mL
Sodium citrate	20 mM
Arginine hydrochloride	140 mM
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 1 is 5.8.

Example 2 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	50 mg/mL
Histidine	20 mM
Arginine hydrochloride	140 mM
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 2 is 5.8.

Example 3 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	50 mg/mL
Sodium citrate	20 mM
Sorbitol	4.5%
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 3 is 5.8.

Example 4 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	25 mg/mL
Sodium citrate	20 mM
Mannitol	4.5%
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 4 is 5.8.

Example 5 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	25 mg/mL
Sodium citrate	20 mM
Sucrose	9%
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 5 is 5.8.

Example 6 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	25 mg/mL
Histidine	20 mM
Sucrose	9%
TWEEN-80	0.04%

The pH of the stable solution formulation of Example 6 is 5.8.

Example 7 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	50 mg/mL
Histidine	20 mM
Arginine hydrochloride	140 mM
Methionine	5 mM
TWEEN-20	0.04%

The pH of the stable solution formulation of Example 7 is 5.8.

Example 8 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	50 mg/mL
Sodium citrate	20 mM
Arginine hydrochloride	140 mM
Methionine	5 mM
TWEEN-20	0.04%

The pH of the stable solution formulation of Example 8 is 5.8.

Example 9 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	100 mg/mL
Sodium citrate	20 mM
Arginine hydrochloride	140 mM

TWEEN-20	0.04%
EDTA	0.05 mM

The pH of the stable solution formulation of Example 9 is 5.8.

Example 10 :

<b>Ingredients</b>	<b>Concentration</b>
ET <sub>A</sub> R antibody A-1	100 mg/mL
Histidine	20 mM
Arginine hydrochloride	140 mM
TWEEN-20	0.04%
EDTA	0.05 mM

The pH of the stable solution formulation of Example 10 is 5.8.

13. Methods for analyzing stable pharmaceutical solution formulations of an ET<sub>A</sub>R antibody:

Size exclusion high performance liquid chromatography (SEC-HPLC) for analyzing monomeric and aggregated ET<sub>A</sub>R antibodies

SEC-HPLC was used to determine the formation of aggregates (soluble aggregates) of the ET<sub>A</sub>R antibody and the loss of its monomeric form. In Agilent 1100 HPLC at 25 °C, a TSK-G3000SWxl high performance SEC column was flushed with 200 mM phosphate buffer (pH 6.8) as a mobile phase till the baseline of the UV absorbance was constant and stable. A stable pharmaceutical solution formulation of the ET<sub>A</sub>R antibody at the concentration of 1 to 3 mg/mL (pre-diluted with the mobile phase) was injected in the amount of 50 µL. The sample was eluted with the mobile phase at a flow rate of 0.5 mL/min and the absorbance at UV 280 nm was recorded. After each run, the AUCs of absorbance peaks of the monomer (the main peak), dimers and multimers were calculated, the percentage of the AUC for the main peak *versus* the total AUC was calculated and reported as the purity of the sample.

Capillary electrophoresis (CE-SDS) for analyzing the purities of reduced and non-reduced ET<sub>A</sub>R antibody samples

A capillary electrophoresis apparatus (Beckmann MDQP/ACE) was used, and samples were treated with an IgG purity/heterogeneity assay kit (Beckmann) by adding

beta-mercaptoethanol to reduced samples or iodoacetamide to non-reduced samples. Beckmann non-coated capillaries were used to separate, and the samples were loaded at a concentration of 1 mg/mL automatically. After finishing loading a sample, separation was performed at 15 kV reverse voltage, and a UV214 nm absorbance time curve was recorded. When the process was finished, the absorption peaks at UV214 nm of the main peak, fragments and aggregates were integrated. For a non-reduced sample, the ratio of the main peak over total area was calculated, which represents the purity of the non-reduced sample. For a reduced sample, the ratio of light and heavy chain peak areas over the total area was calculated, which represents the purity of the reduced sample.

Calcium flux detection method to determine inhibitory activity of an ET<sub>AR</sub> antibody on an ET<sub>AR</sub> *in vitro*

ET<sub>AR</sub>-Aq-#105 cells stably expressing hET<sub>AR</sub>-Aequorin were seeded in a 96 well plate at a density of  $3.5 \times 10^4$  cells/well and 100  $\mu$ L/well, and the plate was placed at 37 °C, 5% CO<sub>2</sub> overnight. 100  $\mu$ L of Colenterazine-h (0.23  $\mu$ M stock solution) was diluted into 3.2 mL of a DMEM/F12 medium without phenol red. The plate was removed from the incubator, the medium was aspirated, 50  $\mu$ L/well of diluted Colenterazine-h was added (in the dark), and the plate was placed in the 37 °C incubator for 2 hours. Reference antibody A-1 and test samples were diluted serially, and 50  $\mu$ L/well of the dilutions were added to the 96 well plate (in the dark). After addition of the samples, the plate was placed in an incubator at 37 °C and 5% CO<sub>2</sub> for another 30 min. The fluorescence intensity of each well was read on a microplate reader. The read values of three wells with only DMEM/F12 medium (no phenol red) were the background values, and the rest of the wells were added with 20 nM endothelin-1.

The read data were pasted to Excel for analysis, and the time point of the peak fluorescent intensity was selected as the calculation value. The average of the fluorescence values of the blank controls was obtained, and the average value was then subtracted from each original value. After processing, the values of the maximum fluorescence intensities were averaged, and the relative percentage of each well to the average of the maximum response average was calculated finally. Through Prism software, the percentage and concentration of each well were used to calculate IC<sub>50</sub> values for the reference ET<sub>AR</sub> antibody and the test samples, as well as curve fitting correlation coefficients R<sup>2</sup>. The

biological activity of a test sample (%) = (IC<sub>50</sub> of the reference / IC<sub>50</sub> of the test sample) x 100.

14. pH and buffer screening:

pH was screened from a pH single factor experiment with antibody A-1 in a 20 mM sodium citrate buffer with pH between 4.7 and 6.3, with a total of 7 experiments (see Table 3) to determine the appearance and A340 absorption of antibody A-1 after 2-hour in a 65 °C water bath. As shown in Table 3, the experimental results indicated that antibody A-1 had relatively good thermal stability within pH 5.0 and 5.7 to 6.3.

Table 3 : Effect of pH on protein thermal stability at 65 °C

Test	pH	A340	Appearance
1	4.7	0.028	Opalescence and large number of floc-like particles
2	5.0	0.073	Floc-like particles
3	5.3	0.451	Large number of particles
4	5.5	0.508	Large number of particles
5	5.7	0.218	Large number of particles
6	6.0	0.036	Floc-like particles
7	6.3	0.012	Floc-like particles

15. Effect of concentrations and types of a buffer salt on protein stability:

Based on the above pH screening result, further screening experiments were carried out on pH and buffers. The two buffer systems of sodium citrate and histidine were selected, and the experiment was designed with two influencing factors. Two sets of samples were prepared to buffer at 3 different pH values, 20 mM sodium citrate, 200 mM sodium chloride, 0.02% TWEEN-80 at pH 4.7, 5.1, and 5.3; 20 mM histidine, 200 mM sodium chloride, 0.02% TWEEN-80 at pH 5.7, 6.0, and 6.3 (*See* Table 4). 1 to 6 groups of accelerated degradation experiments were performed, and the concentration of antibody A-1 was kept at 50 mg/mL. The experimental conditions were freeze-thaw: freeze at -20 °C, thaw at room temperature, 3 cycles; high temperature: 37 °C for 10 days, 13 days; illumination: 5000 lx, 300  $\mu\text{W}/\text{cm}^2$ , 25 °C, 5 days; testing parameters: appearance, visible particles, purity (SEC-HPLC, non-reduced CE-SDS), charge variant.



Table 4 : Experimental design for buffer salt type and pH screening

Formulation	Protein concentration (mg/mL)	Sodium citrate (mM)	Histidine salt (mM)	NaCl (mM)	TWEEN-80 (%)	pH
1	50	20		200	0.02	4.7
2	50	20		200	0.02	5.1
3	50	20		200	0.02	5.3
4	50		20	200	0.02	5.7
5	50		20	200	0.02	6.0
6	50		20	200	0.02	6.3

The results were summarized in Tables 5 to 8. Experimental results indicated that freeze-thaw cycle didn't significantly affect antibody A-1, but based on the results at high temperatures and light exposure, lower pH of sodium citrate resulted readily in the aggregation and loss of purity. Antibody A-1 is relatively sensitive to light, especially in term of charge variant, and after 5 days of light exposure, the main peak dropped significantly and the basic peak decreased. Only formulation 4 changed little, followed by formulation 5. Therefore, formulation 4 was selected as the formulation for the next protectant screening.

Table 5 : Appearance inspection results of formulation samples

Formulation	0 day	High temperature 10 days	High temperature 13 days	Freeze-and-thaw	Illumination 5 days
1	Colorless and clear No visible particles	Slight opalescence No visible particles	Apparent opalescence No visible particles	Colorless and clear No visible particles	Opalescence No visible particles
2	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Opalescence No visible particles
3	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Opalescence No visible particles
4	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Opalescence and slight yellow No visible particles
5	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Slight yellow No visible particles
6	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Colorless and clear No visible particles	Slight yellow No visible particles

Table 6 : SEC purity (%) test results of formulation samples

Formulation	0 day	High temperature 10 days	High temperature 13 days	Freeze-and- thaw	Illumination 5 days
1	98.05	86.32	84.60	98.01	92.11
2	98.01	96.56	95.78	97.95	92.84
3	97.99	97.56	96.72	97.93	93.39
4	98.05	97.85	97.05	98.02	95.12
5	98.04	97.45	97.08	98.02	95.08
6	98.03	97.94	97.16	98.01	94.43

Table 7 : Non-reduced CE-SDS purity (%) test results of formulation samples

Formulation	0 day	High temperature 10 days	High temperature 13 days	Freeze-and- thaw
1	96.50	93.71	92.59	95.80
2	96.78	94.77	89.90	95.81
3	96.40	95.59	95.47	95.76
4	96.20	95.55	95.47	95.77
5	96.12	95.55	95.84	96.13
6	96.22	95.61	95.89	95.21

Table 8 : Changes of charge variant main peak (%) of formulation samples

Formulation	0 day	High temperature 10 days	High temperature 13 days	Freeze-and- thaw
1	59.80	62.53	62.57	58.82
2	59.53	62.42	63.84	59.08
3	59.88	62.64	63.30	58.90
4	59.49	62.09	60.42	58.73
5	59.56	61.17	61.13	59.23
6	60.54	60.76	60.89	59.07

#### 16. Protectant screening:

##### Experimental plan

Two groups of experiments were performed. Six protectants were tested in the first group of experiments, and they are 5% sucrose, 2% mannitol, 2% sorbitol, 100 mM NaCl, 140 mM arginine, and 150 mM proline. In this group, the protein concentration of antibody A-1 was 60 mg/mL, the concentration of histidine as a buffer salt was 20 mM, TWEEN-80 concentration was 0.02%, and pH was 5.8. The experimental conditions were freeze-thaw: freeze at -20 °C, thaw at room temperature, 5 cycles; high temperature: 40 °C for 10 days, 13 days; illumination: 5000 lx, 300  $\mu$ W/cm<sup>2</sup>, 25 °C, 5 days; shaking: 300 rpm, room temperature for 3 days in the dark; testing parameters: appearance, visible particles, purity (SEC-HPLC, non-reduced CE-SDS), charge variant. The design plan is shown in Table 9.

Table 9 : Experimental design for protectant screening

Formulation	NaCl (mM)	Sucrose (%)	Mannitol (%)	Sorbitol (%)	Arginine (mM)	Proline (mM)
7	100	5	--	--	--	--
8	100	--	2	--	--	--
9	100	--	--	2	--	--
10	--	--	--	--	140	--
11	--	--	--	--	--	150
12	100	--	2	--	--	--

The second group of experiments were performed based on the first group of experiments, and sodium citrate was selected as the buffer system. The concentration of sodium citrate was 20 mM, pH was 5.8, and the concentration of antibody A-1 was about 30 mg/mL. In the F1 formulation, arginine was replaced with arginine hydrochloride, and its concentration was 140 mM. Further, the experiment for the selection of a protectant was performed, and conditions of high temperature and illumination were modified to 40 °C/2 watts, 1 month, 5000 lx, 0 day, 2 days, 5 days, 10 days. The design plan is shown in Table 10.

Table 10 : Protectant screening design table

Formulation	Protein Conc. (mg/mL)	Sodium citrate (mM)	Arginine HCl (mM)	Sucrose (%)	Mannitol (%)	Sorbitol (%)	Tween-80 (%)	pH
F1	26.4	20	140	--	--	--	0.04	5.8
F2	30.2	20	--	5	--	--	0.04	5.8
F3	29.4	20	--	--	5	--	0.04	5.8
F4	29.8	20	--	--	--	5	0.04	5.8

### Experimental Results

The results of the first group of experiments showed that, after 5 days of illumination, the color of 6 formulation samples all turned yellow to different degrees. The results of SEC-HPLC indicated, in comparison with other protectants, arginine was more effective in

reducing aggregate formation. (Table 11).

For 7, 13 days at high temperature 40 °C: the results of SEC-HPLC showed, in comparison with the monomer purity (98%) at 0 day, there was no significant difference in the decrease of monomer in each formulation, and there was even less reduction of purity for the formulations containing sucrose, arginine and proline. The results are shown in Table 12 below.

Table 11: Test results of formulation samples after illumination

Formulation	Appearance	Visible particles	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
7	Slight yellow	No visible particles	0.66	4.92	93.66	0.76
8	Slight yellow	No visible particles	0.61	4.77	93.97	0.65
9	Slight yellow	No visible particles	0.62	4.79	93.95	0.64
10	Slight yellow	No visible particles	0.44	3.90	95.15	0.51
11	Slight yellow	No visible particles	0.73	5.07	93.60	0.61
12	Slight yellow	No visible particles	0.66	4.90	93.81	0.63

Table 12: Test results of formulation samples after high temperature

Formulation	Day	Appearance	Visible particle	Monomer (%)	Charge variant main peak ratio (%)
7	7	Colorless and clear	No visible particles	97.88	65.44
8	7	Colorless and clear	No visible particles	97.50	61.50
9	7	Colorless and clear	No visible particles	97.44	63.14
10	7	Colorless and clear	No visible particles	97.77	63.50
11	7	Colorless and clear	No visible particles	97.83	65.24
12	7	Colorless and clear	No visible particles	97.48	63.52
7	13	Colorless and clear	No visible particles	97.40	65.33
8	13	Slight	No visible particles	97.31	61.32

Formulation	Day	Appearance	Visible particle	Monomer (%)	Charge variant main peak ratio (%)
9	13	opalescent	particles	97.15	64.84
		Colorless and clear	No visible particles		
10	13	Colorless	No visible particles	97.34	66.03
		and clear	particles		
11	13	Slight	No visible particles	97.34	65.44
		opalescent	particles		
12	13	Slight	No visible particles	97.25	65.70
		opalescent	particles		

The test results of shaking and freeze-thaw (see Table 13) indicated that, in comparison with 0 day, there was no significant difference for the aggregate and charge variant.

In the second group of experiments, the sodium citrate was used as the buffer, and the results indicates that, after high temperature and illumination, the formulation of antibody A-1 showed slight opalescence (see Table 14). Comparing the effects of several protectants, we considered arginine hydrochloride the best in preserving the purity of antibody A-1 (see Table 15). The illumination induced significant change of charge variants (see Table 16), therefore, it was recommended to store antibody A-1 in the dark.

Table 13: Test results of formulation samples after shaking and freeze-thaw

Formulation	SEC purity (%)			Ratio of charge variant main peak (%)		
	0 day	Shaking	Freeze-thaw	0 day	Shaking	Freeze-thaw
7	97.98	98.03	97.97	60.02	60.24	58.60
8	97.95	98.00	97.88	59.72	59.97	59.07
9	97.99	98.03	97.95	59.51	59.71	58.92
10	98.03	98.08	97.96	59.57	59.65	59.13
11	98.00	98.01	97.97	59.44	60.40	59.22
12	97.99	98.02	97.92	59.39	59.58	58.95

Table 14: Change of appearance and visible particles of formulation samples

Formulation	Appearance		
	0 day	Illumination 10 days	40 °C, 1 month
F1	Clear	Slight opalescence	Slight opalescence
	No visible particles	No visible particles	No visible particles
F2	Clear	Slight opalescence	Slight opalescence
	No visible particles	No visible particles	No visible particles
F3	Clear	Slight opalescence	Slight opalescence
	No visible particles	No visible particles	No visible particles
F4	Clear	Slight opalescence	Slight opalescence
	No visible particles	No visible particles	No visible particles

Table 15: Test results of SEC purities (%) of formulation samples

Formulation	0 day	Shaking	Freeze- thaw	Illumination			High	High
				2 days	5 days	10 days	temperature 2 weeks	temperature 1 month
F1	98.54	98.60	98.54	98.36	98.13	--	98.53	98.53
F2	98.40	98.30	98.44	98.27	97.94	97.83	97.92	96.2
F3	98.36	98.26	96.32	97.96	97.96	97.22	97.94	95.85
F4	98.40	98.30	98.44	98.18	97.83	97.63	97.72	94.92

Note: the sample of illumination for 10 days was left out by mistake, therefore, the SEC purity and charge variant data of the sample was not available.



Table 16: Test results of charge variant main peak (%) of formulation samples

Formulation	0 day	Shaking	Freeze-thaw	Illumination			High	High
				2 days	5 days	10 days	temperature 2 weeks	temperature 1 month
F1	70.40	72.08	72.97	60.34	48.26	--	59.64	56.41
F2	69.64	72.86	72.81	67.96	49.51	47.59	56.86	63.79
F3	72.19	72.71	73.84	65.12	49.56	51.03	69.05	61.88
F4	70.24	72.82	70.2	63.98	49.04	49.54	53.90	61.22

## 17. The experiment on the factors affecting antioxidants:

## Experimental plan

The experiment was to evaluate the protective effect of 0.0187 mg/mL EDTA, and two buffers were selected, 20 mM histidine salt and 20 mM sodium citrate with 0.02% and 0.1% of Tween-80 (Table 17). The concentration of antibody A-1 was 60 mg/mL, arginine hydrochloride was 140 mM, and pH was 5.8. The three formulation samples were subject to accelerated degradation experiment, and the experimental condition was illumination: 5000 lx, 300  $\mu\text{W}/\text{cm}^2$ , 25 °C, 3 days, 6 days; the test items: appearance, visible particles, purity (SEC-HPLC), and charge variant.

Table 17: Antioxidant screening table

Formulation	Histidine salt (mM)	Sodium citrate (mM)	EDTA (mg/mL)	Tween-80 (%)
13	20	--	0.0187	0.02
14	20	--	--	0.1
15	--	20	--	0.02

Based on the above experiment, methionine was added as a screening antioxidant, and the detailed plan was in Table 18. The experiment conditions were: high temperature 40 °C for 2 weeks, 1 month; illumination 5000 lx for 0 day, 2 days, 5 days, 10 days.

Table 18: Methionine formulation screening table

Formulation	Concentration (mg/mL)	Sodium citrate (mM)	Arginine hydrochloride (mM)	Methionine (mM)	Tween-80 (%)	pH
F1-M	~50	20	140	--	0.04	5.8
F2-M	~50	20	140	5	0.04	5.8
F3-M	~50	20	140	10	0.04	5.8

### Experimental results

Based on the results of two groups of experiments, neither of the two protectants affects antibody A-1 significantly. No difference was observed in the different concentrations of Tween used (Table 19, Table 20, Table 21, Table 22), but when using histidine as the formulation buffer salt, the formulation turned yellow after illumination, therefore, sodium citrate is a better choice as the formulation buffer salt. After three days of destruction with illumination, the SEC-HPLC peaks of formulation samples 13 to 15 decreased with no notable difference between them. The peak of the 3-day formulation sample is the same as 0 day's in the dark. After 6 days of destruction by illumination, the peak of sample 13 showed even more reduction, while sample 14 and 15 showed no further reduction. The peak of the 6-day formulation sample is the same as 0 day's in the dark.

Table 19: Test results of formulation samples after illumination

Formulation	Condition	Appearance	Visible particle	SEC purity (%)
13	Illumination 3 days	Slightly yellow	No visible particles	97.11
14	Illumination 3 days	Very slightly yellow	No visible particles	97.27
15	Illumination 3 days	Opalescence	No visible particles	96.97
13	Illumination 3 days	Clear and colorless	No visible particles	98.07
15	Illumination	Clear and colorless	No visible	98.05

Formulation	Condition	Appearance	Visible particle	SEC purity (%)
	3 days		particles	
13	Illumination 6 days	Slightly yellow	Tiny particles	96.12
14	Illumination 6 days	Slightly yellow	Tiny particles	96.41
15	Illumination 6 days	Opalescence	Tiny particles	96.24
13	Illumination 6 days	Clear and colorless	No visible particles	97.97
15	Illumination 6 days	Clear and colorless	No visible particles	98.08

Table 20: Appearance, visible particles and purity change of formulation samples after high temperature 40 °C

Formulation	SEC purity (%)			Appearance		
	0 day	1 week	1 month	0 day	2 weeks	1 month
F1-M	98.51	98.65	97.30	Slight opalescence No visible particles	Opalescence No visible particles	Opalescence No visible particles
F2-M	98.50	98.67	97.37	Slight opalescence No visible particles	Opalescence No visible particles	Opalescence No visible particles
F3-M	98.53	98.66	97.53	Slight opalescence No visible particles	Opalescence No visible particles	Opalescence No visible particles

Table 21: Test results of the SEC purity (%) of formulation samples after illumination and high temperature

Formulation	0 day	Illumination			High	High
		2 days	5 days	10 days	temperature 2 weeks	temperature 1 month
F1-M	98.51	98.23	97.95	97.38	98.65	97.30
F2-M	98.50	98.23	97.98	97.75	98.67	97.37
F3-M	98.53	98.28	98.03	97.85	98.66	97.53

Table 22: Test results of the charge variant main peak (%) of formulation samples after illumination and high temperature

Formulation	0 day	Illumination			High	High
		2 days	5 days	10 days	temperature 2 weeks	temperature 1 month
F1-M	68.83	46.82	47.23	46.39	64.13	55.13
F2-M	70.16	45.53	48.85	45.87	62.43	57.05
F3-M	75.67	62.50	49.97	44.06	64.54	58.48

#### 18. Long-term and accelerated stability study at different protein concentrations:

##### Experimental plan

After determining the initial formulation, we also did long-term and acceleration stability studies on the antibody A-1 at different concentrations. We set the antibody concentrations at 25, 50, and 100 mg/mL, and used the defined formulation to pursue long-term (4 °C, 0 day, 1 month, 2 months, 3 months, 6 months, 9 months) and acceleration (25 °C, 0 day, 1 month, 2 months, 3 months, 6 months, 9 months) stability studies. The test items are: protein concentration, appearance, visible particle, pH, purity (SEC-HPLC, non-reduced CE-SDS), charge variant, bioactivity. The design plan is shown in Table 23 below.

Table 23: Formulation design table for antibody A-1 at different concentrations

Formulation	Protein concentration (mg/mL)	Sodium citrate (mM)	Arginine hydrochloride (mM)	Tween-80 (%)	pH
F11	26.1	20	140	0.04	5.8
F12	53.9	20	140	0.04	5.8
F13	103.1	20	140	0.04	5.8

### Experimental results

From appearance, as the protein concentration increased, the opalescence increased, and it's easier for the formulation samples to turn yellow at high concentration of the antibody (see Table 24). But after nine-month of long-term and acceleration stability test, the formulation sample was still clear and free of visible particles, meeting the requirement of injection. There was no significant change of the protein concentration and pH during the study (see Table 25 and Table 26).

The purity test result indicated, the SEC and CE-SDS purity of antibody A-1 decreased as the time passed and as the concentration increased. The CE-SDS purity of the samples decreased slightly more (see Table 27, Table 28 and Table 29). But after 9-month of stability test, the purity of the product still met the corresponding quality standards and, and the change of purity was within the acceptable range.

The test result of charge variants indicated (see Table 30), the aggregates and acidic/basic charge variants increased as the protein concentration of antibody A-1 increased, and the stability at lower protein concentration is better. However, judging from the shape of the peaks, there is not much difference at the three concentrations, and the trend was consistent. Bioactivity test of the 3-month samples indicated the bioactivity was relatively stable, as shown in Fig. 6 and Fig. 7. Based on the above results, the protein is stable in this formulation for at least 18 months.

Table 24: Appearance change of formulation samples during 0-9 months

Formulation	Temp.	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	Clear	Clear	Clear	Clear	Slight opalescence	Slight opalescence
F12	4 °C	Clear	Clear	Slight opalescence	Slight opalescence	Slight opalescence	Slight opalescence
F13	4 °C	Slight yellow	Slight yellow	Opalescence	Slight yellow and opalescence	Slight yellow and opalescence	Slight yellow and opalescence
F11	25 °C	Clear	Clear	Clear	Clear	Slight opalescence	Slight opalescence
F12	25 °C	Clear	Clear	Slight opalescence	Slight opalescence	Slight opalescence	Slight opalescence
F13	25 °C	Slight yellow	Slight yellow	Opalescence	Slight yellow and opalescence	Slight yellow and opalescence	Slight yellow and opalescence

Table 25: Test results of protein concentrations (mg/mL) of formulation samples in long-term accelerated stability study

Formulations	Temp.	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	26.1	25.7	26.5	27.0	28.0	24.9
F12	4 °C	53.9	54.0	51.6	51.2	54.5	48.4
F13	4 °C	103.1	97.2	101.2	106.7	114.9	94.1
F11	25 °C	26.1	27.7	26.1	27.4	29.5	25.3
F12	25 °C	53.9	51.5	51.7	57.6	58.1	54.3
F13	25 °C	103.1	106.1	99.9	110.1	115.7	94.1

Table 26: Test results of pH of formulation samples in a long-term accelerated stability study

Formulations	Temp.	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	5.89	5.86	5.88	5.87	5.86	5.84
F12	4 °C	5.89	5.86	5.86	5.84	5.86	5.83
F13	4 °C	5.91	5.87	5.88	5.83	5.87	5.86
F11	25 °C	5.89	5.85	5.87	5.96	5.83	5.84
F12	25 °C	5.89	5.86	5.86	5.83	5.88	5.86
F13	25 °C	5.91	5.86	5.88	5.84	5.89	5.87

Table 27: Test results of SEC purity (%) of formulation samples in a long-term accelerated stability study

Formulations	Temp.	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	98.6	98.7	98.7	98.7	98.6	98.7
F12	4 °C	98.6	98.6	98.6	98.7	98.7	98.6
F13	4 °C	98.5	98.6	98.5	98.6	98.6	98.5
F11	25 °C	98.6	98.7	98.7	99.0	98.6	98.1
F12	25 °C	98.6	98.6	98.6	98.8	98.5	98.2
F13	25 °C	98.5	98.5	98.5	98.6	98.1	97.9

Table 28: Test results of the non-reduced CE-SDS purity (%) of formulation samples in a long-term accelerated stability study

Formulations	Temperature	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	97.23	98.26	97.86	97.75	97.88	96.36
F12	4 °C	97.17	98.24	97.78	97.77	96.84	96.01
F13	4 °C	97.06	97.9	97.89	97.39	97.07	96.16
F11	25 °C	97.23	97.42	97.63	96.34	96.68	94.94
F12	25 °C	97.17	97.66	97.51	97.44	96.59	94.80
F13	25 °C	97.06	97.47	97.44	97.42	96.77	94.45

Table 29: Test results of reduced CE-SDS purity (%) of formulation samples in a long-term accelerated stability study

Formulations	Temperature	3 months	6 months	9 months
F11	4 °C	100	100	100
F12	4 °C	100	100	100
F13	4 °C	100	100	99.98
F11	25 °C	100	99.83	99.91
F12	25 °C	99.56	100	99.38
F13	25 °C	99.12	98.49	96.07

Table 30: Test results of the charge variant main peak (%) of formulation samples in a long-term accelerated stability study

Formulations	Temperature	0 day	1 month	2 months	3 months	6 months	9 months
F11	4 °C	74.97	71.40	66.28	72.80	72.30	80.68
F12	4 °C	74.74	71.30	71.01	71.40	67.96	77.81
F13	4 °C	75.01	71.40	65.95	72.40	69.1	77.13
F11	25 °C	74.97	74.48	70.22	66.64	65.69	69.22
F12	25 °C	74.74	75.96	65.47	68.17	65.36	69.26
F13	25 °C	75.01	74.30	69.18	66.97	63.15	78.5

The formulation of antibody A-1 (20 mM sodium citrate, 140 mM arginine hydrochloride, 0.04% TWEEN-80, pH 5.8) was tested at 3 different antibody concentrations in long-term and accelerated stability studies. After 9 months, regardless of acceleration and long-term stability studies, the quality of the protein still met the quality standards set. Especially after 9 months of long-term storage, the purity of antibody A-1 remained above 98%.

The above embodiments are provided to fully disclose and explain how to make and use the claimed embodiments to one of ordinary skill in the art, and they are not meant to limit the scope of this disclosure. Modifications obvious to those skilled in the art are within the scope of the claims herein. All the publications, patents and patent applications



cited in the specifications were incorporated herein as references, just as each of them was specifically and independently incorporated herein as a reference.

## WHAT IS CLAIMED IS:

1. A stable pharmaceutical solution formulation of an ETAR antibody, wherein the formulation comprises an ETAR antibody, a buffer, a surfactant and an amino acid protectant, wherein the formulation has a pH ranging from 5 to 7, and wherein:

the ETAR antibody comprises light chain CDR1 amino acid sequence: SEQ ID NO: 8; light chain CDR2 amino acid sequence: SEQ ID NO: 32; light chain CDR3 amino acid sequence: SEQ ID NO: 50; heavy chain CDR1 amino acid sequence: SEQ ID NO: 70; heavy chain CDR2 amino acid sequence: SEQ ID NO: 92; and heavy chain CDR3 amino acid sequence: SEQ ID NO: 116; and

the ETAR antibody further comprises light chain constant domain amino acid sequence SEQ ID NO: 194 or SEQ ID NO: 196; and heavy chain constant domain amino acid sequence SEQ ID NO: 198; and

wherein the formulation further comprises a surfactant, and an amino acid protectant; wherein the concentration of the ETAR antibody is from 10 to 200 mg/mL; the concentration of the surfactant is from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is from 10 to 200 mM; and the concentration of the buffer is from 1 to 50 mM,

wherein the amino acid protectant comprises arginine hydrochloride,

wherein the buffer comprises sodium citrate, and

wherein the surfactant comprises polysorbate.

2. The formulation of claim 1, wherein the ETAR antibody comprises:

(a) light chain variable domain amino acid sequence SEQ ID NO: 138 and heavy chain variable domain amino acid sequence SEQ ID NO: 166; or

(b) light chain variable domain amino acid sequence SEQ ID NO: 162 and heavy chain variable domain amino acid sequence SEQ ID NO: 190.

3. The formulation of claim 1, wherein the ETAR antibody is a murine ETAR antibody or a humanized ETAR antibody.

4. The formulation of claim 1, wherein the ETAR antibody is a monoclonal ETAR antibody.
5. The formulation of claim 1, wherein concentration of the ETAR antibody is 25 mg/mL, 50 mg/mL, 75 mg/mL, or 100 mg/mL.
6. The formulation of claim 1, wherein the concentration of the buffer is:
  - (a) ranging from 5 mM to 25 mM; or
  - (b) 10 mM, 15 mM, 20 mM, 25 mM, or 30 mM.
7. The formulation of claim 1, wherein the concentration of the surfactant is 0.02, 0.03, 0.04, 0.05, or 0.06 weight/volume percent.
8. The formulation of claim 1, wherein the concentration of the amino acid protectant is 120 mM, 130 mM, 140 mM, 150 mM, or 160 mM.
9. The formulation of claim 1, wherein the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the amino acid protectant is 140 mM; and the concentration of the buffer is 20 mM; and the pH of the formulation is ranging from 5 to 6.
10. The formulation of claim 1, further comprising a polyol protectant.
11. The formulation of claim 10, wherein the polyol protectant comprises:
  - (a) sorbitol, mannitol, sucrose, or trehalose; or
  - (b) sorbitol, mannitol, or sucrose.
12. The formulation of claim 10 or claim 11, wherein the concentration of the polyol protectant is ranging from 0.1 to 50 weight/volume percent, from 1 to 20 weight/volume percent, from 1 to 10 weight/volume percent, or from 4 to 10 weight/volume percent.

13. The formulation of claim 10, wherein:
  - (a) the concentration of the ETAR antibody is ranging from 10 to 200 mg/mL; the concentration of the surfactant is ranging from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is ranging from 1 to 20 weight/volume percent; the concentration of the amino acid protectant is from 10 to 200 mM; and the concentration of the buffer is ranging from 1 to 50 mM; wherein the pH of the formulation is ranging from 5 to 7; or
  - (b) the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the polyol protectant is ranging from 4 to 10 weight/volume percent; and the concentration of the buffer is 20 mM; the concentration of the amino acid protectant is 140 mM; wherein the pH of the formulation is ranging from 5 to 6.
14. The formulation of claim 1, further comprising a metal chelator.
15. The formulation of claim 14, wherein the metal chelator comprises:
  - (a) ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, or ethylene glycol diethyl ether diaminetetraacetic acid; or
  - (b) ethylenediaminetetraacetic acid.
16. The formulation of claim 14 or claim 15, wherein the concentration of the metal chelator is:
  - (a) ranging from 0.001 mM to 1 mM, from 0.005 mM to 0.5 mM;
  - (b) ranging from 0.01 mM to 0.2 mM; or
  - (c) 0.03 mM, 0.04 mM, 0.05 mM, 0.06 mM, or 0.07 mM.
17. The formulation of claim 14, wherein:
  - (a) the concentration of the ETAR antibody is ranging from 10 to 200 mg/mL; the concentration of the metal chelator is ranging from 0.01 mM to 0.2 mM; the concentration of the surfactant is ranging from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is ranging from 10 to 200 mM; and the concentration of the buffer is ranging from 1 to 50 mM; wherein the pH of the formulation is ranging from 5 to 7; or

(b) the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the metal chelator is 0.05 mM; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the amino acid protectant is 140 mM; and the concentration of the buffer is 20 mM; wherein the pH of the formulation is ranging from 5 to 6.

18. The formulation of claim 14, further comprising a polyol protectant.

19. The formulation of claim 18, wherein:

(a) the concentration of the ETAR antibody is ranging from 10 to 200 mg/mL; the concentration of the metal chelator is ranging from 0.01 mM to 0.2 mM; the concentration of the surfactant is ranging from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is ranging from 1 to 20 weight/volume percent; the concentration of the amino acid protectant is from 10 to 200 mM; and the concentration of the buffer is ranging from 1 to 50 mM; wherein the pH of the formulation is ranging from 5 to 7; or

(b) the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the metal chelator is 0.05 mM; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the polyol protectant is ranging from 4 to 10 weight/volume percent; the concentration of the amino acid protectant is 140 mM; and the concentration of the buffer is 20 mM; wherein the pH of the formulation is ranging from 5 to 6.

20. The formulation of claim 1, further comprising an antioxidant.

21. The formulation of claim 20, wherein the antioxidant comprises:

- (a) methionine, vitamin C, thiosulfate, thiosulfate, or benzyl methionine; or
- (b) methionine.

22. The formulation of claim 20 or claim 21, wherein the concentration of the antioxidant is:

- (a) ranging from 0.5 mM to 20 mM;
- (b) ranging from 1 mM to 10 mM; or
- (c) 3 mM, 4 mM, 5 mM, 6 mM, or 7 mM.

23. The formulation of claim 20, wherein:
  - (a) the concentration of the ETAR antibody is ranging from 10 to 200 mg/mL; the concentration of the antioxidant is ranging from 1 mM to 10 mM; the concentration of the surfactant is ranging from 0.01 to 0.1 weight/volume percent; the concentration of the amino acid protectant is ranging from 10 to 200 mM; and the concentration of the buffer is ranging from 1 to 50 mM; wherein the pH of the formulation is ranging from 5 to 7; or
  - (b) the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the antioxidant is 5 mM; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the amino acid protectant is 140 mM; and the concentration of the buffer is 20 mM; wherein the pH of the formulation is ranging from 5 to 6.
24. The formulation of claim 20, further comprising a polyol protectant.
25. The formulation of claim 24, wherein:
  - (a) the concentration of the ETAR antibody is ranging from 10 to 200 mg/mL; the concentration of the antioxidant is ranging from 1 mM to 10 mM; the concentration of the surfactant is ranging from 0.01 to 0.1 weight/volume percent; the concentration of the polyol protectant is ranging from 1 to 20 weight/volume percent; the concentration of the amino acid protectant is ranging from 10 to 200 mM; and the concentration of the buffer is ranging from 1 to 50 mM; wherein the pH of the formulation is ranging from 5 to 7; or
  - (b) the concentration of the ETAR antibody is 25, 50, 75, or 100 mg/mL; the concentration of the antioxidant is 5 mM; the concentration of the surfactant is 0.04 weight/volume percent; the concentration of the polyol protectant is ranging from 4 to 10 weight/volume percent; the concentration of the amino acid protectant is 140 mM; and the concentration of the buffer is 20 mM; wherein the pH of the formulation is ranging from 5 to 6.
26. The formulation of claim 1, wherein the formulation is an aqueous solution.
27. A method of:
  - (a) treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension in a subject;

(b) treating, preventing, or alleviating one or more symptoms of a disease associated with elevated pulmonary arterial pressure in a subject; or

(c) treating, preventing, or alleviating one or more symptoms of cancer of a reproductive organ in a subject;

comprising administering to the subject a therapeutically effective amount of the formulation of any one of claims 1 to 26.

28. Use of the formulation of any one of claims 1 to 26 in the manufacture of a medicament for:

(a) treating, preventing, or alleviating one or more symptoms of pulmonary arterial hypertension in a subject;

(b) treating, preventing, or alleviating one or more symptoms of a disease associated with elevated pulmonary arterial pressure in a subject; or

(c) treating, preventing, or alleviating one or more symptoms of cancer of a reproductive organ in a subject.

29. The method of claim 27 or the use of claim 28, wherein the cancer of a reproductive organ is cancer of a reproductive organ accompanied with elevated pulmonary arterial pressure.

30. The method or use of any one of claims 27 to 29, wherein the subject is a human.

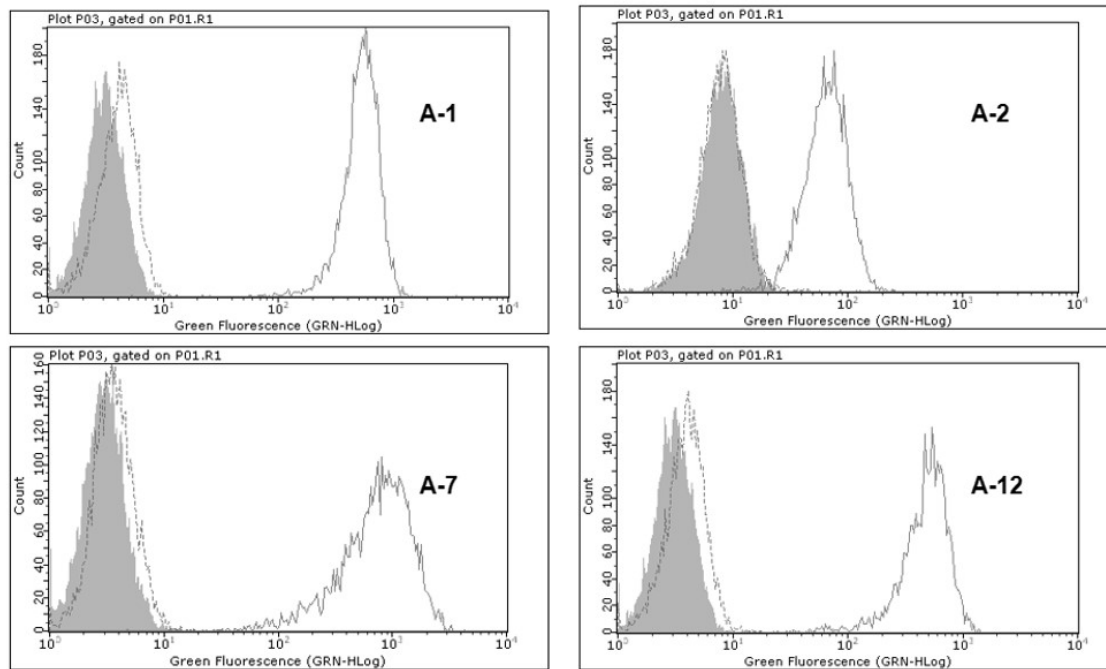
31. The method or use of any one of claims 27 to 30, wherein the formulation is administered intravenously or subcutaneously.

Figure 1

CLONE(#)	CHO-ET <sub>A</sub> R	CHO-DHFR	CLONE(#)	CHO-ET <sub>A</sub> R	CHO-DHFR	CLONE(#)	CHO-ET <sub>A</sub> R	CHO-DHFR
1B4	0.31	0.19	11D11	0.19	0.08	25B5	0.14	0.06
1B7	0.29	0.07	11H12	0.26	0.09	25G8	0.37	0.06
1G8	0.15	0.07	12B1	0.28	0.06	25F9	0.14	0.06
1G12	0.19	0.07	12F1	0.18	0.06	25B10	0.14	0.06
2F5	0.28	0.11	13B9	0.17	0.07	<b>25C11</b>	0.77	0.07
2F12	0.41	0.26	13A12	0.34	0.07	<b>25H10</b>	0.39	0.06
3A1	0.19	0.07	14G6	0.77	0.07	26 E4	0.13	0.07
3 E3	0.16	0.07	14F8	0.16	0.07	27B3	0.16	0.07
3F3	0.16	0.07	14A9	0.34	0.18	27D8	0.35	0.17
3B7	0.16	0.09	14B9	0.37	0.06	27F12	0.18	0.08
3G12	0.19	0.06	<b>14F9</b>	0.90	0.09	28C9	0.22	0.10
4B2	0.35	0.14	14 E10	0.30	0.06	30F3	0.20	0.10
4C7	0.17	0.07	<b>15F3</b>	1.28	0.06	30A5	0.23	0.11
5H1	0.14	0.07	15D7	0.16	0.06	30C11	0.14	0.07
5H3	0.14	0.07	15D10	0.18	0.06	30D11	0.14	0.06
5C12	0.14	0.07	15G11	0.15	0.06	30H12	0.78	0.41
6A1	0.21	0.07	16H4	1.07	0.07	32B7	0.84	0.50
6D2	0.58	0.22	18F1	0.94	0.07	<b>32G12</b>	0.99	0.15
6D5	0.24	0.08	18F3	0.30	0.07	33F1	0.37	0.06
6B6	0.49	0.25	19G12	0.48	0.07	33D6	0.39	0.08
<b>6 E8</b>	1.76	0.41	20H1	0.30	0.06	35G3	0.53	0.07
<b>8 E2</b>	1.33	0.06	21A1	0.91	0.07	<b>35B8</b>	0.61	0.07
<b>8B9</b>	0.36	0.07	21F7	0.15	0.06	36 E9	0.29	0.05
8B10	0.39	0.22	21G12	0.14	0.06	36 E11	0.34	0.06
<b>8C11</b>	0.76	0.08	22A1	0.14	0.06	36F12	1.15	0.06
9D2	0.16	0.07	22C5	0.34	0.17	<b>37B12</b>	0.89	0.08
10A2	0.16	0.07	23G1	0.15	0.06	38A5	1.75	0.08
10C6	0.29	0.07	23D5	0.26	0.13	38F5	1.18	0.07
10B7	0.28	0.07	<b>23 E6</b>	1.13	0.07	40F1	0.70	0.07
10A9	0.14	0.07	24C8	0.17	0.09	<b>40F3</b>	0.47	0.05
11D1	0.25	0.07	24H10	0.17	0.08	<b>40G2</b>	2.22	0.08
11H3	0.16	0.06	24F11	0.14	0.06	40H11	0.22	0.06



Figure 2



	A-1	A-2	A-7	A-12
Relative Fluorescence Intensity	470	105	926	614

Figure 3

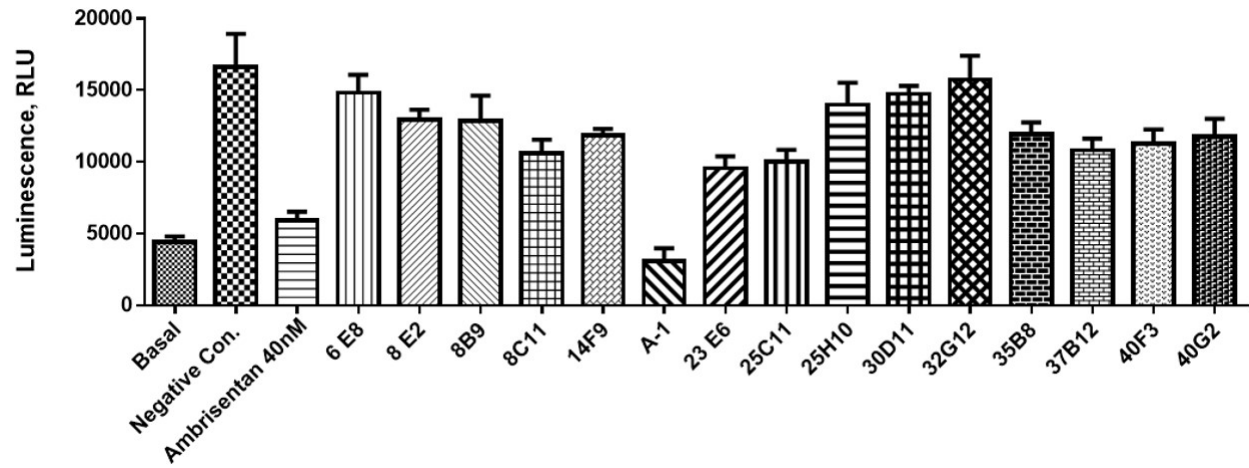
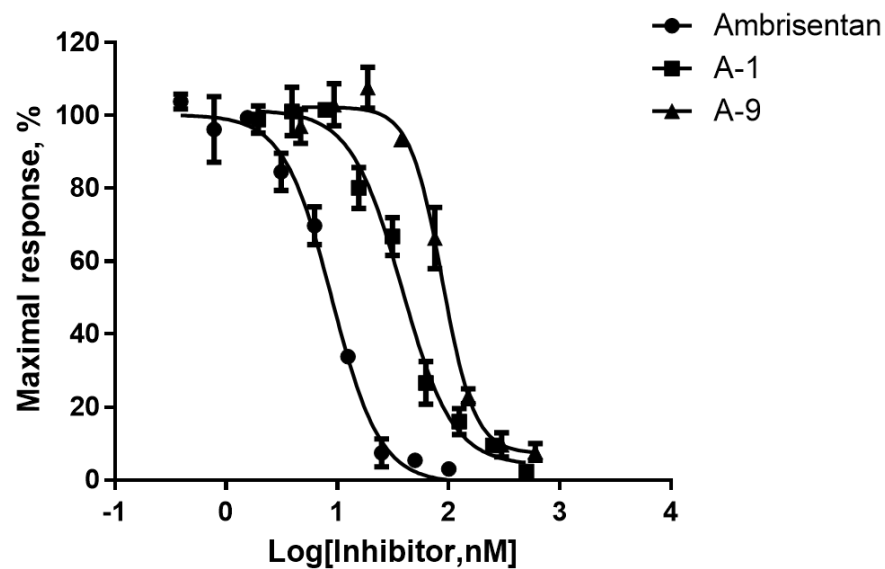


Figure 4



	Ambrisentan	A-1	A-9
IC <sub>50</sub> (nM)	8.95	37.91	87.84
R <sup>2</sup>	0.96	0.97	0.97

Figure 5

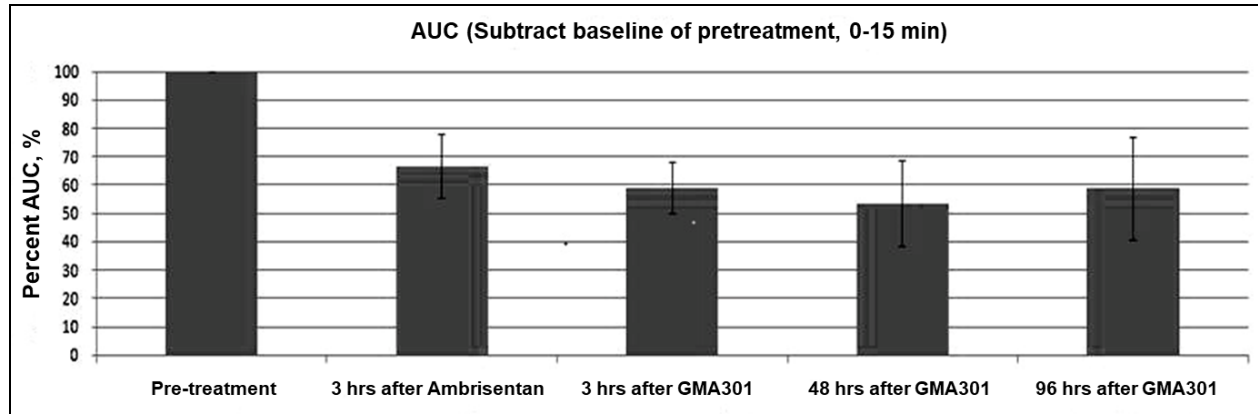


Figure 6

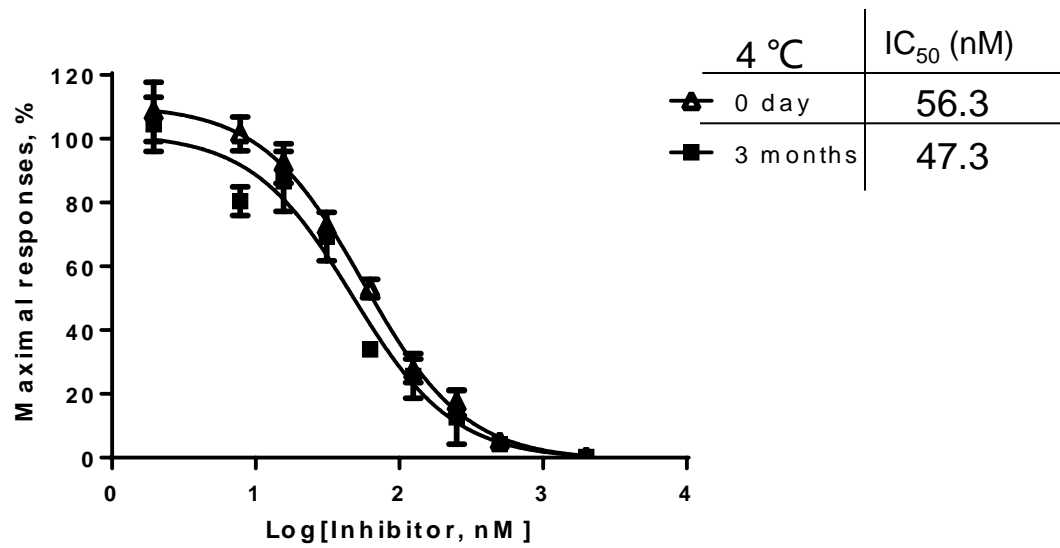
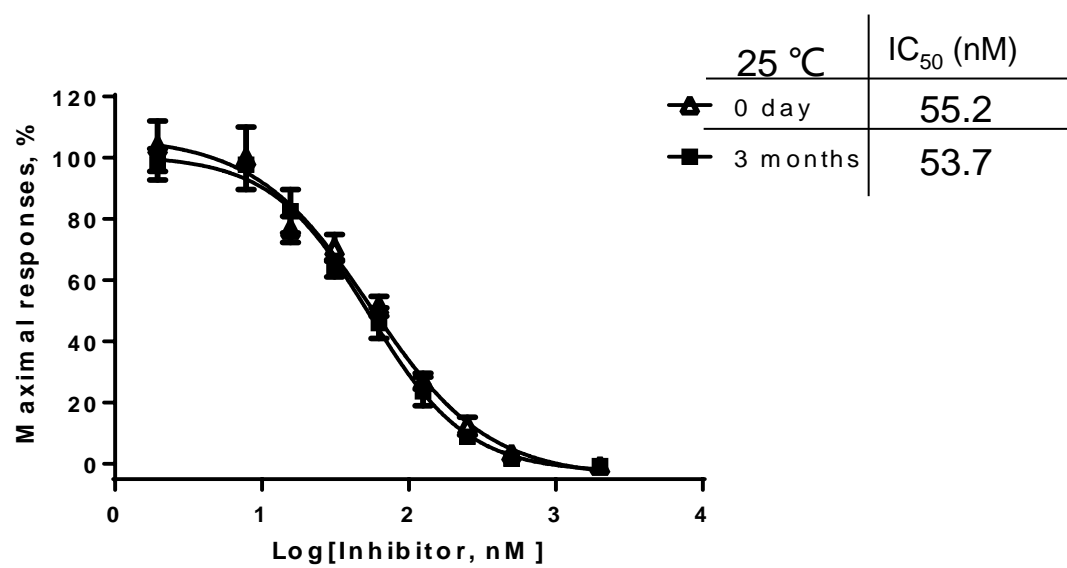


Figure 7



## SEQUENCE LISTING

<110>   °¼ÖY°eÖE»ªÄpÉúÎ ï Ò½Ö©¹¼³Î ÓÐÏ Ð¹«É¼  
 <120>   ETAR¿¹¹ ä£-ÆäÖ©Î ï xé°Î Î ï ¼° ÆäÖ|ÖÄ  
 <130>   2016  
 <160>   204

<170>   PatentIn versi on 3.5

<210>   1  
 <211>   1868  
 <212>   DNA  
 <213>   EE

<400>   1  
 gaattcggga aaaagtgaag gtgtaaaagc agcacaagtg caataagaga tatttcctca        60  
 aatttgcctc aagatggaaa ccctttgcct cagggcatcc ttttggctgg cactgggttg        120  
 atgtgtaatc agtgataatc ctgagagata cagcacaaat ctaagcaatc atgtggatga        180  
 tttcaccact tttcgtggca cagagctcag cttcctgggt accactcatc aaccactaa        240  
 tttggtccta ccagcaatg gctcaatgca caactattgc ccacagcaga ctaaaattac        300  
 ttcagctttc aaatacatta aactgtgat atcttgtact attttcatcg tgggaatggt        360  
 ggggaatgca actctgctca ggatcattta ccagaacaaa tgtatgagga atggccccaa        420  
 cgcgctgata gccagtcttg cccttggaga ccttatctat gtggtcattg atctccctat        480  
 caatgtatit aagctgctgg ctgggcgctg gccttttgat cacaatgact ttggcgtatt        540  
 tctttgcaag ctgttcccct ttttgagaa gtcctcggtg gggatcaccg tcctcaacct        600  
 ctgcgctctt agtgttgaca ggtacagagc agttgcctcc tggagtcgtg ttcagggaat        660  
 tgggattcct ttggtaactg ccattgaaat tgtctccatc tggatcctgt cctttatcct        720  
 ggccattcct gaagcgattg gcttcgtcat ggtacccttt gaatataggg gtgaacagca        780  
 taaaacctgt atgctcaatg ccacatcaaa attcatggag ttctaccaag atgtaaagga        840  
 ctggtggctc ttcgggttct atttctgtat gcccttgggt tgcactgcga tcttctacac        900  
 cctcatgact tgtgagatgt tgaacagaag gaatggcagc ttgagaattg ccctcagtga        960  
 acatcttaag cagcgtcgag aagtggcaaa aacagttttc tgcttggttg taatttttgc        1020  
 tctttgctgg ttccctcttc atttaagccg tatattgaag aaaactgtgt ataacgagat        1080  
 ggacaagaac cgatgtgaat tacttagttt ctactgctc atggattaca tcggtattaa        1140  
 cttggcaacc atgaattcat gtataaacc catagctctg tattttgtga gcaagaaatt        1200  
 taaaaattgt ttccagtcac gcctctgctg ctgctgttac cagtccaaaa gtctgatgac        1260  
 ctcggtcccc atgaacggaa caagcatcca gtggaagaac cacgatcaaa acaaccacaa        1320  
 cacagaccgg agcagccata aggacagcat gaactgacca cccttagaag cactcctcgg        1380  
 tactcccata atcctctcgg agaaaaaaat cacaaggcaa ctgtgagtcc gggaatctct        1440

181708895-seq1

tctctgatcc ttcttcctta attcactccc acacccaaga agaaatgctt tccaaaaccg	1500
caagggtaga ctggtttatc caccacaac atctacgaat cgtacttctt taattgatct	1560
aatttacata ttctgcgtgt tgtattcagc actaaaaaat ggtgggagct gggggagaat	1620
gaagactggt aaatgaaacc agaaggatat ttactacttt tgcataaaaa tagagctttc	1680
aagtacatgg ctagctttta tggcagttct ggtgaatggt caatgggaac tggtcacccat	1740
gaaacttttag agattaacga caagattttc tacttttttt aagtgatttt tttgtccttc	1800
agccaaacac aatatgggct caagtcactt ttatttgaaa tgtcatttgg tgccagtatc	1860
ccgaattc	1868

<210> 2  
 <211> 427  
 <212> PRT  
 <213> EE

<400> 2

Met Glu Thr Leu Cys Leu Arg Ala Ser Phe Trp Leu Ala Leu Val Gly  
 1 5 10 15

Cys Val Ile Ser Asp Asn Pro Glu Arg Tyr Ser Thr Asn Leu Ser Asn  
 20 25 30

His Val Asp Asp Phe Thr Thr Phe Arg Gly Thr Glu Leu Ser Phe Leu  
 35 40 45

Val Thr Thr His Gln Pro Thr Asn Leu Val Leu Pro Ser Asn Gly Ser  
 50 55 60

Met His Asn Tyr Cys Pro Gln Gln Thr Lys Ile Thr Ser Ala Phe Lys  
 65 70 75 80

Tyr Ile Asn Thr Val Ile Ser Cys Thr Ile Phe Ile Val Gly Met Val  
 85 90 95

Gly Asn Ala Thr Leu Leu Arg Ile Ile Tyr Gln Asn Lys Cys Met Arg  
 100 105 110

Asn Gly Pro Asn Ala Leu Ile Ala Ser Leu Ala Leu Gly Asp Leu Ile  
 115 120 125

Tyr Val Val Ile Asp Leu Pro Ile Asn Val Phe Lys Leu Leu Ala Gly  
 130 135 140

Arg Trp Pro Phe Asp His Asn Asp Phe Gly Val Phe Leu Cys Lys Leu  
 145 150 155 160

181708895-seql

Phe Pro Phe Leu Gln Lys Ser Ser Val Gly Ile Thr Val Leu Asn Leu  
 165 170 175  
 Cys Ala Leu Ser Val Asp Arg Tyr Arg Ala Val Ala Ser Trp Ser Arg  
 180 185 190  
 Val Gln Gly Ile Gly Ile Pro Leu Val Thr Ala Ile Glu Ile Val Ser  
 195 200 205  
 Ile Trp Ile Leu Ser Phe Ile Leu Ala Ile Pro Glu Ala Ile Gly Phe  
 210 215 220  
 Val Met Val Pro Phe Glu Tyr Arg Gly Glu Gln His Lys Thr Cys Met  
 225 230 235 240  
 Leu Asn Ala Thr Ser Lys Phe Met Glu Phe Tyr Gln Asp Val Lys Asp  
 245 250 255  
 Trp Trp Leu Phe Gly Phe Tyr Phe Cys Met Pro Leu Val Cys Thr Ala  
 260 265 270  
 Ile Phe Tyr Thr Leu Met Thr Cys Glu Met Leu Asn Arg Arg Asn Gly  
 275 280 285  
 Ser Leu Arg Ile Ala Leu Ser Glu His Leu Lys Gln Arg Arg Glu Val  
 290 295 300  
 Ala Lys Thr Val Phe Cys Leu Val Val Ile Phe Ala Leu Cys Trp Phe  
 305 310 315 320  
 Pro Leu His Leu Ser Arg Ile Leu Lys Lys Thr Val Tyr Asn Glu Met  
 325 330 335  
 Asp Lys Asn Arg Cys Glu Leu Leu Ser Phe Leu Leu Leu Met Asp Tyr  
 340 345 350  
 Ile Gly Ile Asn Leu Ala Thr Met Asn Ser Cys Ile Asn Pro Ile Ala  
 355 360 365  
 Leu Tyr Phe Val Ser Lys Lys Phe Lys Asn Cys Phe Gln Ser Cys Leu  
 370 375 380  
 Cys Cys Cys Cys Tyr Gln Ser Lys Ser Leu Met Thr Ser Val Pro Met  
 385 390 395 400  
 Asn Gly Thr Ser Ile Gln Trp Lys Asn His Asp Gln Asn Asn His Asn  
 405 410 415

## 181708895-seq1

Thr Asp Arg Ser Ser His Lys Asp Ser Met Asn  
 420 425

<210> 3  
 <211> 2696  
 <212> DNA  
 <213> Ðĭ Ěó

<400> 3  
 gtctaggagc ctgtggagtc taaggaagat cgcgggaggc gtgttcctcc ggagtttgct 60  
 ttctcttggg agcctcgcgc gcacacccat cccttctagt ctggcaactg tgtctaggag 120  
 gtggggagcc tctctctgat ccaccggacc atcgctggag cttgcaggct gagcaagatc 180  
 tccccctaga gaagcctggc tgtccgggga agtttccccg agctgagact gtgctgcagc 240  
 cctggtcacc cgccaccctg cgcgccaccc tcgttctcca gctcaggctc cggtggcccc 300  
 gtgcgcggac ctggagctgt ctgcttccga ggagctctaa ggtgaaaaaa agaaaggcgt 360  
 gagaccaaca taagaagact taaaatccag gttaagatga gtatcttttg ccttgcggca 420  
 tacttttggc tgaccatggg gggaggcgta atggctgaca atccggagag atacagcgct 480  
 aatctaagca gccacatgga agacttcacc ccttttccgg ggacggagat caactttctg 540  
 ggcaccaccc atcgaccccc taatttggcc ctgcctagca atggctcaat gcacggctat 600  
 tgcccacagc agactaaaat cacgacagct ttcaaatata ttaacactgt gatatcctgc 660  
 accattttca tcgtgggaat ggtggggaac gcaactctac tacgaatcat ttaccaaacc 720  
 aagtgtatga ggaacggccc caatgcgctc atagccagcc tggcccttgg agaccttacc 780  
 tacgtgggtca ttgacctccc catcaacgtg tttaagctct tggcaggacg ctggcctttc 840  
 gaccacaatg attttggagt gtttctctgc aagctgttcc ccttcctgca gaagtcctcc 900  
 gtgggcatca ccgtcttgaa cctctgtgct ctcatgttgg acaggtacag agcagtggct 960  
 tcctggagcc gagtccaagg aatcgggatc cccttgatta ccgccattga aatcgtctcc 1020  
 atctggattc ttctcttcat ctgggccatc ccggaagcaa tcggcttcgt catggtaccc 1080  
 ttccaataca agggcgagct gcataggacc tgcattgctca acgccacgtc caagttcatg 1140  
 gagttttacc aagatgtgaa ggactggtgg ctctttgggt tctacttctg catgcccttg 1200  
 gtgtgcacag caatcttcta caccctcatg acctgtgaga tgctcaacag gaggaacggc 1260  
 agcttgcgga tcgcccttag tgagcacctc aaacagcgtc gagaagtggc aaagactgtc 1320  
 ttctgcttgg ttgtcatctt cgccctgtgc tggttccctc ttactttaag ccgcattttg 1380  
 aagaaaactg tatatgatga gatggataag aaccggtgtg aactgctcag cttcttgctg 1440  
 ctaatggatt acatcggcat taacctggca accatgaatt cttgcataaa cccaatagct 1500  
 ctatattttg tgagcaagaa attcaaaaaa tgttttcagt cctgcctctg ttgctgttgt 1560



181708895-seq1

caccagtcca aaagcctcat gacctcggtc cccatgaatg gaacgagtat ccagtgggaag	1620
aaccaagagc agaacaacca caacacggaa cggagcagcc acaaggacag catgaactaa	1680
ccctccgcag aaacaccgag acgtgtgcct tcaagtccta ggatggaaac aaccattacg	1740
ccacagatgc gctcccaaaa cctcccaagt ctctcccatg ctctttttct aagtccatcc	1800
taggaaaagc tctcctgccc tcccaacagc acgtgggtgga ccggtcccag ctatagccaa	1860
tgggtctttc ctgagtactg tatatgatatt gcataccgcg catgtcattt ccaacacttg	1920
aaaattagag ctggggagaaa ggagatgatg gttcaaagaa gccacctagc tgccgccttt	1980
gcatgaacac agagtttgca agttcatgac cagcttccgt gcagttctat ggaccagctg	2040
gtgggaactg tccatcctaa gattctagag cagtgggtct caaccttccc aatgctgcag	2100
ccccttaata cagttcttca ttttccagtg acccccccca accacaatat tattttttgt	2160
tgctacttca attattttga attgttataa ttgtctgata tttctgatag tcttagcctg	2220
cccctgttaa agggtcatta gcaacccaca agttgagaac cactgcccta gaaattctgt	2280
tgcgtttcat ggcccatgac tacaatccta aaattggaga ggtgagggaa gatggtcagg	2340
tgttcaaggt tagcctcatc aacatagttc ggaaaagcca gggctacctg ttctcacaag	2400
acacaaacag acaaaaagtg tttcaaagtt atggcagatt cattattatt aattattatt	2460
atcttatagc caaacacatt gtgagggttaa agtactcttt tggaaatgtc accgagtgtt	2520
ggtactttat aactgcatgg taccctagaa atgatcgttt catcttcttt caatgtactc	2580
tgaagaaaag aaataggaga gttccagaag ggagatctgg aaaggagata atgtttgaaa	2640
tgtaaagaag gaaaatatcc aataaaaaaa ttcaaagtct aaaaaaaaaa aaaaaa	2696

<210> 4  
 <211> 427  
 <212> PRT  
 <213> Đj Êó

<400> 4

Met	Ser	Ile	Phe	Cys	Leu	Ala	Ala	Tyr	Phe	Trp	Leu	Thr	Met	Val	Gly
1				5					10					15	

Gly	Val	Met	Ala	Asp	Asn	Pro	Glu	Arg	Tyr	Ser	Ala	Asn	Leu	Ser	Ser
			20					25					30		

His	Met	Glu	Asp	Phe	Thr	Pro	Phe	Pro	Gly	Thr	Glu	Ile	Asn	Phe	Leu
		35					40					45			

Gly	Thr	Thr	His	Arg	Pro	Pro	Asn	Leu	Ala	Leu	Pro	Ser	Asn	Gly	Ser
	50					55					60				

## 181708895-seql

Met His Gly Tyr Cys Pro Gln Gln Thr Lys Ile Thr Thr Ala Phe Lys  
 65 70 75 80  
 Tyr Ile Asn Thr Val Ile Ser Cys Thr Ile Phe Ile Val Gly Met Val  
 85 90  
 Gly Asn Ala Thr Leu Leu Arg Ile Ile Tyr Gln Asn Lys Cys Met Arg  
 100 105 110  
 Asn Gly Pro Asn Ala Leu Ile Ala Ser Leu Ala Leu Gly Asp Leu Ile  
 115 120 125  
 Tyr Val Val Ile Asp Leu Pro Ile Asn Val Phe Lys Leu Leu Ala Gly  
 130 135 140  
 Arg Trp Pro Phe Asp His Asn Asp Phe Gly Val Phe Leu Cys Lys Leu  
 145 150 155 160  
 Phe Pro Phe Leu Gln Lys Ser Ser Val Gly Ile Thr Val Leu Asn Leu  
 165 170 175  
 Cys Ala Leu Ser Val Asp Arg Tyr Arg Ala Val Ala Ser Trp Ser Arg  
 180 185 190  
 Val Gln Gly Ile Gly Ile Pro Leu Ile Thr Ala Ile Glu Ile Val Ser  
 195 200 205  
 Ile Trp Ile Leu Ser Phe Ile Leu Ala Ile Pro Glu Ala Ile Gly Phe  
 210 215 220  
 Val Met Val Pro Phe Glu Tyr Lys Gly Glu Leu His Arg Thr Cys Met  
 225 230 235 240  
 Leu Asn Ala Thr Ser Lys Phe Met Glu Phe Tyr Gln Asp Val Lys Asp  
 245 250 255  
 Trp Trp Leu Phe Gly Phe Tyr Phe Cys Met Pro Leu Val Cys Thr Ala  
 260 265 270  
 Ile Phe Tyr Thr Leu Met Thr Cys Glu Met Leu Asn Arg Arg Asn Gly  
 275 280 285  
 Ser Leu Arg Ile Ala Leu Ser Glu His Leu Lys Gln Arg Arg Glu Val  
 290 295 300  
 Ala Lys Thr Val Phe Cys Leu Val Val Ile Phe Ala Leu Cys Trp Phe  
 305 310 315 320

181708895-seq1

Pro Leu His Leu Ser Arg Ile Leu Lys Lys Thr Val Tyr Asp Glu Met  
325 330 335

Asp Lys Asn Arg Cys Glu Leu Leu Ser Phe Leu Leu Leu Met Asp Tyr  
340 345 350

Ile Gly Ile Asn Leu Ala Thr Met Asn Ser Cys Ile Asn Pro Ile Ala  
355 360 365

Leu Tyr Phe Val Ser Lys Lys Phe Lys Asn Cys Phe Gln Ser Cys Leu  
370 375 380

Cys Cys Cys Cys His Gln Ser Lys Ser Leu Met Thr Ser Val Pro Met  
385 390 395 400

Asn Gly Thr Ser Ile Gln Trp Lys Asn Gln Glu Gln Asn Asn His Asn  
405 410 415

Thr Glu Arg Ser Ser His Lys Asp Ser Met Asn  
420 425

<210> 5  
<211> 1436  
<212> DNA  
<213> 5'GAG

<400> 5  
gtgagaccaa cataacagga cgtttcttca gatccacatt aagatgggtg tcctttgctt 60  
tctggcgctc ttttggctgg ccctgggtggg aggcgcaatc gctgacaatg ctgagagata 120  
cagtgctaata ctaagcagcc acgtggagga cttcaccctt tttccagggga cagagttcga 180  
ctttctgggc accacccttc gaccccttaa tttggccctg cctagcaatg gctcaatgca 240  
tggctattgc ccacagcaga caaaaatcac gacggctttc aaatatatca aactgtgat 300  
atcctgtacc attttcatcg tgggaatggt ggggaacgcc actctcctaa gaatcattta 360  
ccaaaacaag tgtatgagga acggcccca tgcgctcata gccagcctgg cccttggaga 420  
ccttatctac gtggctattg atctcccat caatgtgttt aagctgttgg cggggcgctg 480  
gccttttgac cacaatgatt ttggagtgtt tctctgcaag ctgttcccct ttttgcagaa 540  
gtcgtccgtg ggcatactg tcctgaatct ctgcgctctc agtgtggaca ggtacagagc 600  
agtggcttcc tggagccggg ttcaaggaat cgggatcccc ttgattaccg ccattgaaat 660  
tgtctccatc tggatccttt cctttatctt ggccatccca gaagcaatcg gcttcgtcat 720  
ggtacccttc gaatacaagg gcgagcagca caggacctgc atgctcaacg ccacgaccaa 780  
gttcatggag ttttaccaag acgtgaagga ctggtggctc tttggattct acttctgcat 840

181708895-seq1

```

gcccttggtg tgcacagcaa tcttctatac cctcatgacc tgtgagatgc tcaacagaag      900
gaatgggagc ttgcggattg ccttcagcga acacctcaag cagcgtcgag aggtggcaaa      960
gaccgtcttc tgcttggttg tcattcttcgc cctgtgctgg ttccctcttc acttaagccg    1020
aattttgaag aaaaccgtct atgatgagat ggataagaac cgggtgtgaac tgctcagctt    1080
cttgctgctc atggattaca ttggcattaa cctggcaacc atgaactctt gcataaacc    1140
aatagctctg tattttgtga gcaagaaatt caaaaattgt ttccagtcac gcctctgttg    1200
ctgttgtcac cagtccaaaa gcctcatgac ctcgggtcccc atgaatggaa cgagtatcca    1260
gtggaagaac caggagcaga accacaacac agaacggagc agccacaagg acagcatgaa    1320
ctaaccctgt gcagaagcac cgagcagtgt gccttcgagt cccaggatga aacggtcacg    1380
cagcagctgc gctcccaaaa cctcccaggt ctctcccctg ctttttgtct aagctt      1436

```

<210> 6  
 <211> 426  
 <212> PRT  
 <213> 'óĖó

<400> 6

Met Gly Val Leu Cys Phe Leu Ala Ser Phe Trp Leu Ala Leu Val Gly  
 1 5 10 15

Gly Ala Ile Ala Asp Asn Ala Glu Arg Tyr Ser Ala Asn Leu Ser Ser  
 20 25 30

His Val Glu Asp Phe Thr Pro Phe Pro Gly Thr Glu Phe Asp Phe Leu  
 35 40 45

Gly Thr Thr Leu Arg Pro Pro Asn Leu Ala Leu Pro Ser Asn Gly Ser  
 50 55 60

Met His Gly Tyr Cys Pro Gln Gln Thr Lys Ile Thr Thr Ala Phe Lys  
 65 70 75 80

Tyr Ile Asn Thr Val Ile Ser Cys Thr Ile Phe Ile Val Gly Met Val  
 85 90 95

Gly Asn Ala Thr Leu Leu Arg Ile Ile Tyr Gln Asn Lys Cys Met Arg  
 100 105 110

Asn Gly Pro Asn Ala Leu Ile Ala Ser Leu Ala Leu Gly Asp Leu Ile  
 115 120 125

Tyr Val Val Ile Asp Leu Pro Ile Asn Val Phe Lys Leu Leu Ala Gly  
 130 135 140

181708895-seql

Arg Trp Pro Phe Asp His Asn Asp Phe Gly Val Phe Leu Cys Lys Leu  
 145 150 155 160  
 Phe Pro Phe Leu Gln Lys Ser Ser Val Gly Ile Thr Val Leu Asn Leu  
 165 170 175  
 Cys Ala Leu Ser Val Asp Arg Tyr Arg Ala Val Ala Ser Trp Ser Arg  
 180 185 190  
 Val Gln Gly Ile Gly Ile Pro Leu Ile Thr Ala Ile Glu Ile Val Ser  
 195 200 205  
 Ile Trp Ile Leu Ser Phe Ile Leu Ala Ile Pro Glu Ala Ile Gly Phe  
 210 215 220  
 Val Met Val Pro Phe Glu Tyr Lys Gly Glu Gln His Arg Thr Cys Met  
 225 230 235 240  
 Leu Asn Ala Thr Thr Lys Phe Met Glu Phe Tyr Gln Asp Val Lys Asp  
 245 250 255  
 Trp Trp Leu Phe Gly Phe Tyr Phe Cys Met Pro Leu Val Cys Thr Ala  
 260 265 270  
 Ile Phe Tyr Thr Leu Met Thr Cys Glu Met Leu Asn Arg Arg Asn Gly  
 275 280 285  
 Ser Leu Arg Ile Ala Leu Ser Glu His Leu Lys Gln Arg Arg Glu Val  
 290 295 300  
 Ala Lys Thr Val Phe Cys Leu Val Val Ile Phe Ala Leu Cys Trp Phe  
 305 310 315 320  
 Pro Leu His Leu Ser Arg Ile Leu Lys Lys Thr Val Tyr Asp Glu Met  
 325 330 335  
 Asp Lys Asn Arg Cys Glu Leu Leu Ser Phe Leu Leu Leu Met Asp Tyr  
 340 345 350  
 Ile Gly Ile Asn Leu Ala Thr Met Asn Ser Cys Ile Asn Pro Ile Ala  
 355 360 365  
 Leu Tyr Phe Val Ser Lys Lys Phe Lys Asn Cys Phe Gln Ser Cys Leu  
 370 375 380  
 Cys Cys Cys Cys His Gln Ser Lys Ser Leu Met Thr Ser Val Pro Met  
 385 390 395 400

181708895-seq1

Asn Gly Thr Ser Ile Gln Trp Lys Asn Gln Glu Gln Asn His Asn Thr  
 405 410 415

Glu Arg Ser Ser His Lys Asp Ser Met Asn  
 420 425

<210> 7  
 <211> 33  
 <212> DNA  
 <213> Ðí Ěó

<400> 7  
 agggccagtc agaacattgg cacaagcata cac

33

<210> 8  
 <211> 11  
 <212> PRT  
 <213> Ðí Ěó

<400> 8

Arg Ala Ser Gln Asn Ile Gly Thr Ser Ile His  
 1 5 10

<210> 9  
 <211> 33  
 <212> DNA  
 <213> Ðí Ěó

<400> 9  
 cgagcaagtg aaaatatatta cagttattta gca

33

<210> 10  
 <211> 11  
 <212> PRT  
 <213> Ðí Ěó

<400> 10

Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Leu Ala  
 1 5 10

<210> 11  
 <211> 39  
 <212> DNA  
 <213> Ðí Ěó

<400> 11  
 cagagcctct ttgatattga tggaaagaca tatttgaat

39

<210> 12  
 <211> 13  
 <212> PRT  
 <213> Ðí Ěó

181708895-seq1

<400> 12

Gln Ser Leu Phe Asp Ile Asp Gly Lys Thr Tyr Leu Asn  
1 5 10

<210> 13

<211> 33

<212> DNA

<213> Ðí Ěó

<400> 13

cgggcaagtc aggacattgg tggtagctta aac

33

<210> 14

<211> 11

<212> PRT

<213> Ðí Ěó

<400> 14

Arg Ala Ser Gln Asp Ile Gly Gly Ser Leu Asn  
1 5 10

<210> 15

<211> 33

<212> DNA

<213> Ðí Ěó

<400> 15

agggccagcc agactattag cgacttctta cac

33

<210> 16

<211> 11

<212> PRT

<213> Ðí Ěó

<400> 16

Arg Ala Ser Gln Thr Ile Ser Asp Phe Leu His  
1 5 10

<210> 17

<211> 33

<212> DNA

<213> Ðí Ěó

<400> 17

agggcaagtg aggacatata cactcaatta gcc

33

<210> 18

<211> 11

<212> PRT

<213> Ðí Ěó

<400> 18

181708895-seq1  
 Arg Ala Ser Glu Asp Ile His Thr Gln Leu Ala  
 1 5 10

<210> 19  
 <211> 48  
 <212> DNA  
 <213> Ðí Ěó

<400> 19  
 agatctagtc agtacattgt tcatagtact ggaaccacct atttagaa 48

<210> 20  
 <211> 16  
 <212> PRT  
 <213> Ðí Ěó

<400> 20  
 Arg Ser Ser Gln Tyr Ile Val His Ser Thr Gly Thr Thr Tyr Leu Glu  
 1 5 10 15

<210> 21  
 <211> 48  
 <212> DNA  
 <213> Ðí Ěó

<400> 21  
 agatctagtc attaccttgt tcatgataac ggaaacacct atgttgaa 48

<210> 22  
 <211> 16  
 <212> PRT  
 <213> Ðí Ěó

<400> 22  
 Arg Ser Ser His Tyr Leu Val His Asp Asn Gly Asn Thr Tyr Val Glu  
 1 5 10 15

<210> 23  
 <211> 48  
 <212> DNA  
 <213> Ðí Ěó

<400> 23  
 agatctagtc agaacattgt ccatagtact ggaaacacct atttagaa 48

<210> 24  
 <211> 16  
 <212> PRT  
 <213> Ðí Ěó

<400> 24  
 Arg Ser Ser Gln Asn Ile Val His Ser Thr Gly Asn Thr Tyr Leu Glu  
 1 5 10 15



181708895-seq1

<210> 25  
 <211> 30  
 <212> DNA  
 <213> Ðí Ěó

<400> 25  
 agtgtcagct caagtgaag ttacatacac

30

<210> 26  
 <211> 10  
 <212> PRT  
 <213> Ðí Ěó

<400> 26

Ser Val Ser Ser Ser Val Ser Tyr Ile His  
 1 5 10

<210> 27  
 <211> 30  
 <212> DNA  
 <213> Ðí Ěó

<400> 27  
 agtgccagct caagtgaag ttacatgtgc

30

<210> 28  
 <211> 10  
 <212> PRT  
 <213> Ðí Ěó

<400> 28

Ser Ala Ser Ser Ser Val Ser Tyr Met Cys  
 1 5 10

<210> 29  
 <211> 18  
 <212> DNA  
 <213> Ðí Ěó

<400> 29  
 cagggcatta acaattat

18

<210> 30  
 <211> 6  
 <212> PRT  
 <213> Ðí Ěó

<400> 30

Gln Gly Ile Asn Asn Tyr  
 1 5

<210> 31  
 <211> 21

181708895-seq1

<212> DNA  
<213> Ðí Êó

<400> 31  
tatgcttcta agtctatatc t

21

<210> 32  
<211> 7  
<212> PRT  
<213> Ðí Êó

<400> 32  
Tyr Ala Ser Lys Ser Ile Ser  
1 5

<210> 33  
<211> 21  
<212> DNA  
<213> Ðí Êó

<400> 33  
aatgcaaaaa ccttagcaga a

21

<210> 34  
<211> 7  
<212> PRT  
<213> Ðí Êó

<400> 34  
Asn Ala Lys Thr Leu Ala Glu  
1 5

<210> 35  
<211> 21  
<212> DNA  
<213> Ðí Êó

<400> 35  
ctggtgtctg aattggactc t

21

<210> 36  
<211> 7  
<212> PRT  
<213> Ðí Êó

<400> 36  
Leu Val Ser Glu Leu Asp Ser  
1 5

<210> 37  
<211> 21  
<212> DNA  
<213> Ðí Êó

<400> 37  
gccacatcca gcttagattc t

21

<210> 38  
<211> 7  
<212> PRT  
<213> Ðĭ Ėó

<400> 38

Al a Thr Ser Ser Leu Asp Ser  
1 5

<210> 39  
<211> 21  
<212> DNA  
<213> Ðĭ Ėó

<400> 39  
tatgcttccc aatccatctc t

21

<210> 40  
<211> 7  
<212> PRT  
<213> Ðĭ Ėó

<400> 40

Tyr Al a Ser Gln Ser Ile Ser  
1 5

<210> 41  
<211> 21  
<212> DNA  
<213> Ðĭ Ėó

<400> 41  
ggtgcagcca gtttgaaaag t

21

<210> 42  
<211> 7  
<212> PRT  
<213> Ðĭ Ėó

<400> 42

Gly Al a Al a Ser Leu Lys Ser  
1 5

<210> 43  
<211> 21  
<212> DNA  
<213> Ðĭ Ėó

<400> 43  
aaagtttcca accgattttc t

21

181708895-seq1

<210> 44  
 <211> 7  
 <212> PRT  
 <213> Ðĭ Ėó

<400> 44

Lys Val Ser Asn Arg Phe Ser  
 1 5

<210> 45  
 <211> 21  
 <212> DNA  
 <213> Ðĭ Ėó

<400> 45

gacacatcca aactggcttc t

21

<210> 46  
 <211> 7  
 <212> PRT  
 <213> Ðĭ Ėó

<400> 46

Asp Thr Ser Lys Leu Al a Ser  
 1 5

<210> 47  
 <211> 21  
 <212> DNA  
 <213> Ðĭ Ėó

<400> 47

tatacatcaa ctttacagtc a

21

<210> 48  
 <211> 7  
 <212> PRT  
 <213> Ðĭ Ėó

<400> 48

Tyr Thr Ser Thr Leu Gl n Ser  
 1 5

<210> 49  
 <211> 27  
 <212> DNA  
 <213> Ðĭ Ėó

<400> 49

caacatagtt atagcttccc gtggacg

27

<210> 50  
 <211> 9

181708895-seq1

<212> PRT  
<213> Ðí Ěó

<400> 50

Gln His Ser Tyr Ser Phe Pro Trp Thr  
1 5

<210> 51  
<211> 27  
<212> DNA  
<213> Ðí Ěó

<400> 51  
cagcatcatt atggtattcc gttcacg

27

<210> 52  
<211> 9  
<212> PRT  
<213> Ðí Ěó

<400> 52

Gln His His Tyr Gly Ile Pro Phe Thr  
1 5

<210> 53  
<211> 27  
<212> DNA  
<213> Ðí Ěó

<400> 53  
tggcaaggta cacattttcc gctcacg

27

<210> 54  
<211> 9  
<212> PRT  
<213> Ðí Ěó

<400> 54

Trp Gln Gly Thr His Phe Pro Leu Thr  
1 5

<210> 55  
<211> 27  
<212> DNA  
<213> Ðí Ěó

<400> 55  
ctacaatatg ctagttctcc gtatagc

27

<210> 56  
<211> 9  
<212> PRT  
<213> Ðí Ěó

&lt;400&gt; 56

Leu Gln Tyr Ala Ser Ser Pro Tyr Thr  
 1 5

&lt;210&gt; 57

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Ðí Ėó

&lt;400&gt; 57

caaagtggta acacctttcc gtggacg

27

&lt;210&gt; 58

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Ðí Ėó

&lt;400&gt; 58

Gln Ser Gly Asn Thr Phe Pro Trp Thr  
 1 5

&lt;210&gt; 59

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Ðí Ėó

&lt;400&gt; 59

caacagtata ggagtattcc gtggacg

27

&lt;210&gt; 60

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Ðí Ėó

&lt;400&gt; 60

Gln Gln Tyr Arg Ser Ile Pro Trp Thr  
 1 5

&lt;210&gt; 61

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Ðí Ėó

&lt;400&gt; 61

tttcaaggtt cacattttcc attcacg

27

&lt;210&gt; 62

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Ðí Ėó

&lt;400&gt; 62

Phe Gln Gly Ser His Phe Pro Phe Thr

1

5

<210> 63  
 <211> 27  
 <212> DNA  
 <213> Ðí Ěó

<400> 63  
 tttcaagggtt cacatttccc attcacg

27

<210> 64  
 <211> 9  
 <212> PRT  
 <213> Ðí Ěó

<400> 64

Hi s Gln Trp Ser Thr Asn Pro Pro Thr  
 1 5

<210> 65  
 <211> 27  
 <212> DNA  
 <213> Ðí Ěó

<400> 65  
 cagcagtgga gtagtaaccc acccacg

27

<210> 66  
 <211> 9  
 <212> PRT  
 <213> Ðí Ěó

<400> 66

Gln Gln Trp Ser Ser Asn Pro Pro Thr  
 1 5

<210> 67  
 <211> 24  
 <212> DNA  
 <213> Ðí Ěó

<400> 67  
 cagcagttta gtaaacttcg gaca

24

<210> 68  
 <211> 8  
 <212> PRT  
 <213> Ðí Ěó

<400> 68

Gln Gln Phe Ser Lys Leu Arg Thr  
 1 5

181708895-seq1

<210> 69  
<211> 36  
<212> DNA  
<213> Ðí Ěó

<400> 69  
gggttctcac tgaccacttc tggcttgggt gttgcc

36

<210> 70  
<211> 12  
<212> PRT  
<213> Ðí Ěó

<400> 70

Gly Phe Ser Leu Thr Thr Ser Gly Leu Gly Val Ala  
1 5 10

<210> 71  
<211> 30  
<212> DNA  
<213> Ðí Ěó

<400> 71  
ggctacacct ttactagcta ctggatacac

30

<210> 72  
<211> 10  
<212> PRT  
<213> Ðí Ěó

<400> 72

Gly Tyr Thr Phe Thr Ser Tyr Trp Ile His  
1 5 10

<210> 73  
<211> 30  
<212> DNA  
<213> Ðí Ěó

<400> 73  
ggcctcaaca ttaaagacat ctatattcac

30

<210> 74  
<211> 10  
<212> PRT  
<213> Ðí Ěó

<400> 74

Gly Leu Asn Ile Lys Asp Ile Tyr Ile His  
1 5 10

<210> 75  
<211> 30  
<212> DNA



## 181708895-seq1

&lt;213&gt; Ðí Êó

&lt;400&gt; 75

ggttactcat tcaccaacta ctggatacac

30

&lt;210&gt; 76

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Ðí Êó

&lt;400&gt; 76

Gly	Tyr	Ser	Phe	Thr	Asn	Tyr	Trp	Ile	His
1				5					10

&lt;210&gt; 77

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Ðí Êó

&lt;400&gt; 77

ggattcactt tcagtgacta tcccatgtct

30

&lt;210&gt; 78

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Ðí Êó

&lt;400&gt; 78

Gly	Phe	Thr	Phe	Ser	Asp	Tyr	Pro	Met	Ser
1				5					10

&lt;210&gt; 79

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Ðí Êó

&lt;400&gt; 79

ggattcactt tcagtagctt tggcatgtct

30

&lt;210&gt; 80

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Ðí Êó

&lt;400&gt; 80

Gly	Phe	Thr	Phe	Ser	Ser	Phe	Gly	Met	Ser
1				5					10

&lt;210&gt; 81

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Ðí Êó

&lt;400&gt; 81

ggattcactt tcagtaccta tggcatgtct 181708895-seql 30

<210> 82  
 <211> 10  
 <212> PRT  
 <213> Ðí Ěó

<400> 82

Gly Phe Thr Phe Ser Thr Tyr Gly Met Ser  
 1 5 10

<210> 83  
 <211> 30  
 <212> DNA  
 <213> Ðí Ěó

<400> 83  
 ggattcactt tcagtagtta tggcatgtct 30

<210> 84  
 <211> 10  
 <212> PRT  
 <213> Ðí Ěó

<400> 84

Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser  
 1 5 10

<210> 85  
 <211> 36  
 <212> DNA  
 <213> Ðí Ěó

<400> 85  
 gggttttcac tgaccacttc tggtatgggt gtaggc 36

<210> 86  
 <211> 12  
 <212> PRT  
 <213> Ðí Ěó

<400> 86

Gly Phe Ser Leu Thr Thr Ser Gly Met Gly Val Gly  
 1 5 10

<210> 87  
 <211> 36  
 <212> DNA  
 <213> Ðí Ěó

<400> 87  
 ggattttcac tgagcacttc tggtttgggt gtaggc 36

181708895-seq1

<210> 88  
 <211> 12  
 <212> PRT  
 <213> Ðí Êó

<400> 88

Gly Phe Ser Leu Ser Thr Ser Gly Leu Gly Val Gly  
 1 5 10

<210> 89  
 <211> 24  
 <212> DNA  
 <213> Ðí Êó

<400> 89

ggattcacct tcagtgatta ttac

24

<210> 90  
 <211> 8  
 <212> PRT  
 <213> Ðí Êó

<400> 90

Gly Phe Thr Phe Ser Asp Tyr Tyr  
 1 5

<210> 91  
 <211> 48  
 <212> DNA  
 <213> Ðí Êó

<400> 91

cacatttggg cggatgggtga cacgcgctat taccagccc tgaagaac

48

<210> 92  
 <211> 16  
 <212> PRT  
 <213> Ðí Êó

<400> 92

His Ile Trp Ser Asp Gly Asp Thr Arg Tyr Tyr Pro Ala Leu Lys Asn  
 1 5 10 15

<210> 93  
 <211> 36  
 <212> DNA  
 <213> Ðí Êó

<400> 93

tacattaatc ctgacactga ttatagtgag tacaat

36

<210> 94  
 <211> 12  
 <212> PRT

181708895-seq1

<213> Ðí Ěó

<400> 94

Tyr Ile Asn Pro Asp Thr Asp Tyr Ser Glu Tyr Asn  
1 5 10

<210> 95

<211> 36

<212> DNA

<213> Ðí Ěó

<400> 95

aggattgatc ctgcgaacgg taagactgca tatgac

36

<210> 96

<211> 12

<212> PRT

<213> Ðí Ěó

<400> 96

Arg Ile Asp Pro Ala Asn Gly Lys Thr Ala Tyr Asp  
1 5 10

<210> 97

<211> 36

<212> DNA

<213> Ðí Ěó

<400> 97

atgattgatc cttccgatgc tgaaactggg ttaaatt

36

<210> 98

<211> 12

<212> PRT

<213> Ðí Ěó

<400> 98

Met Ile Asp Pro Ser Asp Ala Glu Thr Gly Leu Asn  
1 5 10

<210> 99

<211> 24

<212> DNA

<213> Ðí Ěó

<400> 99

gttagtgatg gtggtgggtc cacc

24

<210> 100

<211> 8

<212> PRT

<213> Ðí Ěó

<400> 100

181708895-seq1

Val Ser Asp Gly Gly Gly Ser Thr  
1 5

<210> 101  
<211> 24  
<212> DNA  
<213> Ðí Êó

<400> 101  
attagtagtg ctggtagttt cacc

24

<210> 102  
<211> 8  
<212> PRT  
<213> Ðí Êó

<400> 102

Ile Ser Ser Ala Gly Ser Phe Thr  
1 5

<210> 103  
<211> 51  
<212> DNA  
<213> Ðí Êó

<400> 103  
accattaata ctaatggtgg taccacctat tatcgagaca gtgtgaaggg c

51

<210> 104  
<211> 17  
<212> PRT  
<213> Ðí Êó

<400> 104

Thr Ile Asn Thr Asn Gly Gly Thr Thr Tyr Tyr Arg Asp Ser Val Lys  
1 5 10 15

Gly

<210> 105  
<211> 51  
<212> DNA  
<213> Ðí Êó

<400> 105  
accataaata ctaatggtgg taacacctat tattcagaca atgtgaaggg c

51

<210> 106  
<211> 17  
<212> PRT  
<213> Ðí Êó

181708895-seq1

<400> 106

Thr Ile Asn Thr Asn Gly Gly Asn Thr Tyr Tyr Ser Asp Asn Val Lys  
1 5 10 15

Gly

<210> 107

<211> 51

<212> DNA

<213> Ðí Êó

<400> 107

accattagta ctaatggtgc caccgccaat tatccagaca gtgtgaaggg c

51

<210> 108

<211> 17

<212> PRT

<213> Ðí Êó

<400> 108

Thr Ile Ser Thr Asn Gly Ala Thr Ala Asn Tyr Pro Asp Ser Val Lys  
1 5 10 15

Gly

<210> 109

<211> 48

<212> DNA

<213> Ðí Êó

<400> 109

cacatttggg gggatgatga taagtactat aatccatccc tgaagagc

48

<210> 110

<211> 16

<212> PRT

<213> Ðí Êó

<400> 110

His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser Leu Lys Ser  
1 5 10 15

<210> 111

<211> 48

<212> DNA

<213> Ðí Êó

<400> 111

cacatttggg gggatgatga taagtactat aatccatccc ttaagaga

48

181708895-seq1

<210> 112  
 <211> 16  
 <212> PRT  
 <213> Ðí Êó

<400> 112

His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser Leu Lys Arg  
 1 5 10 15

<210> 113  
 <211> 30  
 <212> DNA  
 <213> Ðí Êó

<400> 113

attagaaatc gggctaattg ttacacaaca

30

<210> 114  
 <211> 10  
 <212> PRT  
 <213> Ðí Êó

<400> 114

Ile Arg Asn Arg Ala Asn Gly Tyr Thr Thr  
 1 5 10

<210> 115  
 <211> 30  
 <212> DNA  
 <213> Ðí Êó

<400> 115

atgaaggatg atagtcttta ctttgacaac

30

<210> 116  
 <211> 10  
 <212> PRT  
 <213> Ðí Êó

<400> 116

Met Lys Asp Asp Ser Leu Tyr Phe Asp Asn  
 1 5 10

<210> 117  
 <211> 30  
 <212> DNA  
 <213> Ðí Êó

<400> 117

gcaagtgctg gttattatit ttttgacttc

30

<210> 118  
 <211> 10  
 <212> PRT

181708895-seq1

<213> Ðí Ěó

<400> 118

Al a Ser Al a Gl y Tyr Tyr Phe Phe Asp Phe  
1 5 10

<210> 119

<211> 15

<212> DNA

<213> Ðí Ěó

<400> 119

ggtagggggg cccac

15

<210> 120

<211> 5

<212> PRT

<213> Ðí Ěó

<400> 120

Gl y Arg Gl y Al a Hi s  
1 5

<210> 121

<211> 33

<212> DNA

<213> Ðí Ěó

<400> 121

gcaagaattg gcgattacta taatatggac tac

33

<210> 122

<211> 11

<212> PRT

<213> Ðí Ěó

<400> 122

Al a Arg Il e Gl y Asp Tyr Tyr Asn Met Asp Tyr  
1 5 10

<210> 123

<211> 45

<212> DNA

<213> Ðí Ěó

<400> 123

acaagacatg cttcctacta tagctacgac cattctatgg actac

45

<210> 124

<211> 15

<212> PRT

<213> Ðí Ěó

<400> 124



181708895-seq1

Thr Arg His Ala Ser Tyr Tyr Ser Tyr Asp His Ser Met Asp Tyr  
1 5 10 15

<210> 125  
<211> 36  
<212> DNA  
<213> Ðí Êó

<400> 125  
gcaagacggg ggtacgacgt tgggtgcttt gaccac

36

<210> 126  
<211> 12  
<212> PRT  
<213> Ðí Êó

<400> 126

Ala Arg Arg Gly Tyr Asp Val Gly Cys Phe Asp His  
1 5 10

<210> 127  
<211> 27  
<212> DNA  
<213> Ðí Êó

<400> 127  
gcaagagact acggggctat ggactac

27

<210> 128  
<211> 9  
<212> PRT  
<213> Ðí Êó

<400> 128

Ala Arg Asp Tyr Gly Ala Met Asp Tyr  
1 5

<210> 129  
<211> 27  
<212> DNA  
<213> Ðí Êó

<400> 129  
gcaactgaaa agggagctat gggctac

27

<210> 130  
<211> 9  
<212> PRT  
<213> Ðí Êó

<400> 130

Ala Thr Glu Lys Gly Ala Met Gly Tyr  
1 5

181708895-seq1

<210> 131  
<211> 57  
<212> DNA  
<213> Ðí Ěó

<400> 131  
gctcgaagaa ctgagactat gattacgaca gtgctatatt actatgctat ggactac 57

<210> 132  
<211> 19  
<212> PRT  
<213> Ðí Ěó

<400> 132

Al a Arg Arg Thr Gl u Thr Met Ile Thr Thr Val Leu Tyr Tyr Tyr Al a  
1 5 10 15

Met Asp Tyr

<210> 133  
<211> 54  
<212> DNA  
<213> Ðí Ěó

<400> 133  
gctcgaagga gggaagttaa cttcggtatt aactattact attctatgga ctac 54

<210> 134  
<211> 18  
<212> PRT  
<213> Ðí Ěó

<400> 134

Al a Arg Arg Arg Gl u Val Asn Phe Gly Ile Asn Tyr Tyr Tyr Ser Met  
1 5 10 15

Asp Tyr

<210> 135  
<211> 36  
<212> DNA  
<213> Ðí Ěó

<400> 135  
gtaagagatt cctatcacta cgggtacttc gatgtc 36

<210> 136  
<211> 12  
<212> PRT  
<213> Ðí Ěó

181708895-seql

<400> 136

Val Arg Asp Ser Tyr His Tyr Gly Tyr Phe Asp Val  
1 5 10

<210> 137

<211> 324

<212> DNA

<213> Ðí Ěó

<400> 137

gacatcttgc tgactcagtc tccagccatc ctgtctgtga gtccaggaaa aagagtcagt 60  
ttctcctgca gggccagtca gaacattggc acaagcatat actggtatca gcaaagaaca 120  
aatggttctc caaggcttct cataaagtat gcttctaagt ctatatctgg gatttcttcc 180  
aggttttagtg gcagtggctc agggacagat ttactctta gtatcaacag tgtggagtct 240  
gaagatattg cagcttatta ctgtcaacat agttatagct tcccgtggac gttcgggtgga 300  
ggcaccaagc tggaaatcaa acgg 324

<210> 138

<211> 108

<212> PRT

<213> Ðí Ěó

<400> 138

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

Lys Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Asn Ile Gly Thr Ser  
20 25 30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile  
35 40 45

Lys Tyr Ala Ser Lys Ser Ile Ser Gly Ile Ser Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser  
65 70 75 80

Glu Asp Ile Ala Ala Tyr Tyr Cys Gln His Ser Tyr Ser Phe Pro Trp  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg  
100 105

<210> 139

<211> 324

## 181708895-seq1

<212> DNA  
 <213> Ðí Ěó

<400> 139  
 gacatccaga tgactcagtc tccagcctcc ctatctacat ctgtgggaga aactgtcacc 60  
 atcacatgtc gagcaagtga aaatatttac agttatttag catggatatca gcagagacag 120  
 ggaaaatctc ctcacctcct ggtcaataat gcaaaaacct tagcagaagg tgtgccatca 180  
 aggttcagtg gcagtggatc aggcacacat ttttctctga ggatcagcgg cctgcagcct 240  
 gaagattttg ggagttatta ctgtcagcat cattatggta ttccgttcac gttcggaggg 300  
 gggaccaagt tgtcaataaa acgg 324

<210> 140  
 <211> 108  
 <212> PRT  
 <213> Ðí Ěó

<400> 140

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

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr  
 20 25 30

Leu Ala Trp Tyr Gln Gln Arg Gln Gly Lys Ser Pro His Leu Leu Val  
 35 40 45

Asn Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr His Phe Ser Leu Arg Ile Ser Gly Leu Gln Pro  
 65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Gly Ile Pro Phe  
 85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Ser Ile Lys Arg  
 100 105

<210> 141  
 <211> 339  
 <212> DNA  
 <213> Ðí Ěó

<400> 141  
 gatgttgtga tgaccagat tccactcact ttgtcggtta ccattggaca accagcctcc 60  
 atctcttgca agtcaagtca gagcctcttt gatattgatg gaaagacata tttgaattgg 120  
 ttgttacaga ggccaggcca gtctccaaag cgctaattct atctggtgtc tgaattggac 180

181708895-seq1

tctggagtcc ctgacagggt cactggcagt ggatcaggga cagatttcac actgaaaatc 240  
 agcagagtgg aggctgagga tttgggagtt tactattggt ggcaaggtag acattttccg 300  
 ctcacgttcg gtgctggggac caagctggag ctgaaacgg 339

<210> 142  
 <211> 113  
 <212> PRT  
 <213> Ðĭ Ėó

<400> 142

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Phe Asp Ile  
 20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser  
 35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Glu Leu Asp Ser Gly Val Pro  
 50 55 60

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

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Trp Gln Gly  
 85 90 95

Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 100 105 110

Arg

<210> 143  
 <211> 324  
 <212> DNA  
 <213> Ðĭ Ėó

<400> 143

gacatccaga tgacccagtc tccatcctcc ttatctgcct ctctgggaga aagagtcagt 60  
 ctcaattgtc gggcaagtca ggacattggt ggtagcttaa actggcttca gcagaaacca 120  
 gatggaacta ttaaacgcct gatctacgcc acatccagct tagattctgg tgtcccaaaa 180  
 aggttcagtg gcagtaggtc tgggtcagtt ttttctctca ccatcaccag ccttgagtct 240  
 gaagattttg tagactatit ctgtctacaa tatgctagtt ctccgtatac gttcggaggg 300

gggaccaagc tggaaataaa acgg

324

<210> 144  
 <211> 108  
 <212> PRT  
 <213> Ðí Ěó

&lt;400&gt; 144

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

Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Asp Ile Gly Gly Ser  
 20 25 30

Leu Asn Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg Leu Ile  
 35 40 45

Tyr Ala Thr Ser Ser Leu Asp Ser Gly Val Pro Lys Arg Phe Ser Gly  
 50 55 60

Ser Arg Ser Gly Ser Val Phe Ser Leu Thr Ile Thr Ser Leu Glu Ser  
 65 70 75 80

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

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105

<210> 145  
 <211> 324  
 <212> DNA  
 <213> Ðí Ěó

&lt;400&gt; 145

gacattgtga tgactcagtc tccagccacc ctgtctgtga ctccaggaga tagagtctct 60

ctttcctgca gggccagcca gactattagc gacttcttac actggtatca acaaaaatca 120

catgagtctc caaggcttct catcaaatat gcttcccaat ccatctctgg gatcccctcc 180

aggttcagtg gcactggatc agggtcagat ttactctca ctatcaacag tgtggaacct 240

gaagatgttg gagtgtatta ctgtcaaagt ggtaacacct ttccgtggac gttcgggtgga 300

ggcaccaagc tggaaatcaa acgg 324

<210> 146  
 <211> 108  
 <212> PRT  
 <213> Ðí Ěó

&lt;400&gt; 146

181708895-seq1

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

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

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile  
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly  
50 55 60

Thr Gly Ser Gly Ser Asp Phe Thr Leu Thr Ile Asn Ser Val Glu Pro  
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Ser Gly Asn Thr Phe Pro Trp  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg  
100 105

<210> 147  
<211> 324  
<212> DNA  
<213> Ðĭ Ėó

<400> 147  
gacatccaga tgacacaatc ttcatcctcc ttttctggat ttctaggaga cagagtcacc 60  
attacttgca gggcaagtga ggacatacac actcaattag cctgggtatca gcagaaacca 120  
ggaaatgctc ctaggctctt aatatctggt gcagccagtt tgaaaagtgg ggttccttca 180  
agattcagtg gcaactggatc tggaaaaggat tacactctca gcattaccag tcttcagact 240  
gaagatgttg ctacatatta ctgtcaacag tataggagta ttccgtggac gttcgggtgga 300  
ggcaccaagc tggaaatcaa acgg 324

<210> 148  
<211> 108  
<212> PRT  
<213> Ðĭ Ėó

<400> 148

Asp Ile Gln Met Thr Gln Ser Ser Ser Ser Phe Ser Gly Phe Leu Gly  
1 5 10 15

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

181708895-seq1

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

Ser Gly Ala Ala Ser Leu Lys Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Thr Gly Ser Gly Lys Asp Tyr Thr Leu Ser Ile Thr Ser Leu Gln Thr  
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Arg Ser Ile Pro Trp  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg  
100 105

<210> 149  
<211> 339  
<212> DNA  
<213> Ðĭ Ėó

<400> 149  
gatgttttga tgacccaaac tccgctctcc ctgcctgtca gtcttggaga tcacgcctcc 60  
atctcttgca gatctagtca gtacattggt catagtactg gaaccaccta tttagaatgg 120  
tacctacaga aaccaggcca gtctccacag ctctgatct acaaagtttc caaccgattt 180  
tctgggggtcc cagacagggt cactggcagt ggatcaggga cagatttcac actcaggatc 240  
agcagagtgg aggctgagga tctgggaggt tatttctgct ttcaagggtc acattttcca 300  
ttcacgttcg gctcggggac aaagttggaa ataaaacgg 339

<210> 150  
<211> 113  
<212> PRT  
<213> Ðĭ Ėó

<400> 150

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

Asp His Ala Ser Ile Ser Cys Arg Ser Ser Gln Tyr Ile Val His Ser  
20 25 30

Thr Gly Thr Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

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

Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile  
Page 36



65 70 75 80

181708895-seq1

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Phe Gln Gly

85 90 95

Ser His Phe Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys

100 105 110

<210>	151	
<211>	339	
<212>	DNA	
<213>	Đj Ěó	
<400>	151	
gaagttgtga	tgacccaaac tccactctcc ttgcctgtca gtcttggaga tcaagcctcc	60
atctcttgca	gatctagtca ttaccttggt catgataacg gaaacaccta tgttgaatgg	120
tacctgcaga	agccaggcca gtctccaaag ctctgatct acaagggttc caaccgattt	180
tctggagtcc	cagacagggt tactggcagt ggttcaggga cagatttcac actcaagatc	240
agcagagtgg	agtctgagga tctgggaatt tattactgct ttcaagggtc acatttcccc	300
ttcacgttcg	gctcgggggac agagttggaa ataaaacgg	339

<400> 152

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

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

Asn Gly Asn Thr Tyr Val Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

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

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

Ser Arg Val Glu Ser Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly  
85 90 95

181708895-seq1

Ser His Phe Pro Phe Thr Phe Gly Ser Gly Thr Glu Leu Glu Ile Lys  
100 105 110

Arg

<210> 153  
<211> 339  
<212> DNA  
<213> ÐĵĖó

<400> 153  
gatgttttga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60  
atctcttgca gatctagtca gaacattgtc catagtactg gaaacaccta tttagaatgg 120  
tacctgcaga aaccaggcca gtctccaaag ctcttgattt ataaagtttc caaccgattt 180  
tctgggggtcc caaacagggt ccgtggcagt ggatcagga cagatttcac actcaagatc 240  
accagagtgg aggctgagga tctgggaatt tattactgct ttcaaggttc acattttcca 300  
ttcacgttcg gctcggggac aaagttggaa ataaaacgg 339

<210> 154  
<211> 113  
<212> PRT  
<213> ÐĵĖó

<400> 154

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

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser  
20 25 30

Thr Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

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

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

Thr Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly  
85 90 95

Ser His Phe Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys  
100 105 110

181708895-seql

Arg

<210> 155  
<211> 321  
<212> DNA  
<213> Ðĭ Ėó

<400> 155  
caaattgttc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc 60  
atgacctgca gtgtcagctc aagtgttaagt tacatacact ggtaccaaca gaagtcaggc 120  
acctccccca aaagatggat ttatgacaca tccaaaactgg cttctggagt ccctgctcgc 180  
ttcagtggca gtgggtctgg gacctcttac tctctcaca tcagcagcat ggaggctgaa 240  
gatgctgcca cttattactg ccaccagtgg agtactaacc caccacggt cgaggggggg 300  
accaagctgg aaataagacg g 321

<210> 156  
<211> 107  
<212> PRT  
<213> Ðĭ Ėó

<400> 156  
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
1 5 10 15  
Glu Lys Val Thr Met Thr Cys Ser Val Ser Ser Ser Val Ser Tyr Ile  
20 25 30  
His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr  
35 40 45  
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
50 55 60  
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu  
65 70 75 80  
Asp Ala Ala Thr Tyr Tyr Cys His Gln Trp Ser Thr Asn Pro Pro Thr  
85 90 95  
Phe Gly Gly Gly Thr Lys Leu Glu Ile Arg Arg  
100 105

<210> 157  
<211> 321  
<212> DNA  
<213> Ðĭ Ėó

181708895-seq1

<400> 157  
 caaatgttc tcaccagtc tccagcactc atgtctgcat ctccagggga gaaggtcacc 60  
 atgacctgca gtgccagctc aagtgtaatg tacatgtgct ggtaccagca gaagccaaga 120  
 tcctcccca aaccctggat ttatctcaca tccaacctgg cttctggagt ccctgctcgc 180  
 ttcagtggca gtgggtctgg gacctcttac tctctcaca tcagtagcat ggaggctgaa 240  
 gatgctgcca cttattactg ccagcagtg agtagtaacc caccacgtt cgggtgctggg 300  
 accaagctgg agctgaaacg g 321

<210> 158  
 <211> 107  
 <212> PRT  
 <213> Ðĭ Ėó

<400> 158  
 Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly  
 1 5 10 15  
 Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met  
 20 25 30  
 Cys Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr  
 35 40 45  
 Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu  
 65 70 75 80  
 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr  
 85 90 95  
 Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg  
 100 105

<210> 159  
 <211> 321  
 <212> DNA  
 <213> Ðĭ Ėó

<400> 159  
 gaaatccaga tgacacagac tccatcctcc ctgtctgcct ctctgggaga cagagtcacc 60  
 atcacttgca gtgcaagtca gggcattaac aattatttga actggtatca gcagaaacca 120  
 ggtggaaaga ctagactcct catctattat acatcaactt tacagtcagg agtcccatca 180  
 aggttcagtg gcagtgggtc tgggacacat tattctctca ccatcagcaa tctggaacct 240

181708895-seq1

gaagatattg ccacttacta ttgtcagcag tttagtaaac ttcggacatt cggaggaggc 300  
accaggctgg aaatcaaacg g 321

<210> 160  
<211> 107  
<212> PRT  
<213> Ðĭ Ėó  
  
<400> 160

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Gly Ile Asn Asn Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Gly Lys Thr Arg Leu Leu Ile  
35 40 45

Tyr Tyr Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr His Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro  
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Phe Ser Lys Leu Arg Thr  
85 90 95

Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Arg  
100 105

<210> 161  
<211> 324  
<212> DNA  
<213> ĖĖ  
  
<220>  
<221> ¿ĖĬ »±äĬ »ÖĀ  
<222> (128). . (128)  
<223> μŪ128Ĭ »μĀX¿Ė00ĖÇc»ða

<400> 161  
gagattgtgc tgactcagag tccagacttc cagtcagtga cccccaagga gaaagtcacc 60  
atcacatgcc gggcaagcca gaacatcggc acaagcattc actggtacca gcagaagccc 120  
gatcagtxcc ctaagctgct gatcaaatat gcctctaaga gtatttcagg ggtgccctct 180  
agattcagcg gctccggggtc tggaacagac ttactctga ccattaactc cctggaggct 240  
gaagatgccg ctacttacta ttgtcagcat agctactcat tcccttggac attcgggcag 300  
gggaccaaaag tggaaatcaa acgt 324

181708895-seql

<210> 162  
<211> 108  
<212> PRT  
<213> EE

<220>  
<221> ¿ÉÍ »±äÎ »ÖÃ  
<222> (43) . (43)  
<223> µU43Î »µAXaa¿É00ÊÇSer»òTyr

<400> 162

Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys  
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Asn Ile Gly Thr Ser  
20 25 30

Ile His Trp Tyr Gln Gln Lys Pro Asp Gln Xaa Pro Lys Leu Leu Ile  
35 40 45

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

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala  
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Tyr Ser Phe Pro Trp  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
100 105

<210> 163  
<211> 339  
<212> DNA  
<213> EE

<400> 163

gatattgtga tgacccaaac tccgctctcc ctgtccgtca cccctggaca gccggcctcc	60
atctcttgca gatctagtca gaacattgtt catagtactg gaaacaccta tttagaatgg	120
tacctacaga aaccaggcca gtctccacag ctctgatct acaaagtttc caaccgattt	180
tctgggggcc cagacagggt cagtggcagt ggatcaggga cagatttcac actcaaaatc	240
agcagagtgg aggctgagga tgttggagtt tattactgct ttcaagggtc acattttcca	300
ttcacgttcg gccaaaggac caagggtggaa atcaaactg	339

<210> 164  
<211> 113

181708895-seql

<212> PRT

<213> EE

<400> 164

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser  
20 25 30

Thr Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

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

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly  
85 90 95

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

Arg

<210> 165

<211> 360

<212> DNA

<213> Dj Éó

<400> 165

caggttactc tgaaagagtc tggccctggg atattgcagc cctcccagac cctcagtctg 60

acttgttctt tctctgggtt ctactgacc acttctggct tgggtgttgc ctggattcgt 120

cagccttcag ggaagggctt ggagtggctg gcacacattt ggtcggatgg tgacacgcgc 180

tattaccag cctgaagaa ccgactgaca atctccaagg attcctccag caaccaggtc 240

ttcctcaaga tcgcccgtgt ggacactgca gatactgcca catactactg tgctcgaatg 300

aaggatgata gtctttactt tgacaactgg ggccaaggca ctattttcac agtctcctca 360

<210> 166

<211> 120

<212> PRT

<213> Dj Éó

<400> 166

181708895-seq1

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln  
1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Thr Thr Ser  
20 25 30

Gly Leu Gly Val Ala Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu  
35 40 45

Trp Leu Ala His Ile Trp Ser Asp Gly Asp Thr Arg Tyr Tyr Pro Ala  
50 55 60

Leu Lys Asn Arg Leu Thr Ile Ser Lys Asp Ser Ser Ser Asn Gln Val  
65 70 75 80

Phe Leu Lys Ile Ala Arg Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala Arg Met Lys Asp Asp Ser Leu Tyr Phe Asp Asn Trp Gly Gln  
100 105 110

Gly Thr Ile Phe Thr Val Ser Ser  
115 120

<210> 167  
<211> 351  
<212> DNA  
<213> Ðí Ěó

<400> 167  
cagatccagt tgggtgcagtc tggggctgaa ctggcaaaac ctggggcctc agtgaggatg 60  
tcctgcgaga ctctgggcta cacctttact agctactgga tacactggat aaaagagagg 120  
cctggacagg gtctggaatg gattggatac attaatcctg aactgatta tagtgagtac 180  
aatcagaaat tcaaggacaa ggccagattg actgcagaca aatcctccac cacagcctac 240  
atggagctga acagcctgac atttgatgat tctgcagtct attactgtgc aagtgtcgtt 300  
tattatTTTT ttgacttctg gggccaaggc accactctca cagtctcctc a 351

<210> 168  
<211> 117  
<212> PRT  
<213> Ðí Ěó

<400> 168

Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Leu Ala Lys Pro Gly Ala  
1 5 10 15

Ser Val Arg Met Ser Cys Glu Thr Ser Gly Tyr Thr Phe Thr Ser Tyr



181708895-seql

20

25

30

Trp Ile His Trp Ile Lys Glu Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Tyr Ile Asn Pro Asp Thr Asp Tyr Ser Glu Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Arg Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr  
65 70 75 80

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

Ala Ser Ala Gly Tyr Tyr Phe Phe Asp Phe Trp Gly Gln Gly Thr Thr  
100 105 110

Leu Thr Val Ser Ser  
115

<210> 169  
<211> 336  
<212> DNA  
<213> Ðí Ěó

<400> 169  
gaggttcagc tgcagcagtc tggggcagaa cttgtgaaac caggggcctc agtcaagttg 60  
tcctgtacaa cttctggcct caacattaaa gacatctata ttactgggt gaagcagagg 120  
cctgaacagg gcctggagtg gattgggagg attgatcctg cgaacggtaa gactgcatat 180  
gacctgaagt tccaggccaa ggccactata acagcagaca catcttccaa aacagcctac 240  
ctgcacctca gcagcctgac atctgaggac actgccgtct attactgtgg tagggggggcc 300  
cactggggcc aaggcaccac tctcacagtc tcctca 336

<210> 170  
<211> 112  
<212> PRT  
<213> Ðí Ěó

<400> 170

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

Ser Val Lys Leu Ser Cys Thr Thr Ser Gly Leu Asn Ile Lys Asp Ile  
20 25 30

Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile  
35 40 45

181708895-seq1

Gly Arg Ile Asp Pro Ala Asn Gly Lys Thr Ala Tyr Asp Leu Lys Phe  
50 55 60

Gln Ala Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Lys Thr Ala Tyr  
65 70 75 80

Leu His Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Gly Arg Gly Ala His Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser  
100 105 110

<210> 171  
<211> 354  
<212> DNA  
<213> Ðĭ Ėó

<400> 171  
cagatccagt tgggtgcagtc tgggcctcag ctggttaggc ctggggcttc agtgaagata 60  
tcctgcgagg ctcttggtta ctattcacc aactactgga tacactgggt gaagcagagg 120  
cctggacagg gtcttgagtg gattggcatg attgatcctt ccgatgctga aactgggtta 180  
aatcagaagt tcaaggacaa ggccacattg actgtagaca aatcctccag cacagcctac 240  
atgcaactca gcagcccgcac atctgaagac tctgcggtct attactgtgc aagaattggc 300  
gattactata atatggacta ctgggggtcaa ggaacctcag tcaccgtctc ctca 354

<210> 172  
<211> 118  
<212> PRT  
<213> Ðĭ Ėó

<400> 172

Gln Ile Gln Leu Val Gln Ser Gly Pro Gln Leu Val Arg Pro Gly Ala  
1 5 10 15

Ser Val Lys Ile Ser Cys Glu Ala Ser Gly Tyr Ser Phe Thr Asn Tyr  
20 25 30

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

Gly Met Ile Asp Pro Ser Asp Ala Glu Thr Gly Leu Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80

181708895-seq1

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

Ala Arg Ile Gly Asp Tyr Tyr Asn Met Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Ser Val Thr Val Ser Ser  
115

<210> 173  
<211> 366  
<212> DNA  
<213> Ðj Êó

<400> 173  
gaagtgaagg tgggtggagtc tgggggaggt ttagtgcagc ctggaggggc cctgaaactc 60  
tcctgtgcag cctctggatt cactttcagt gactatccca tgtcttgggt tcgccagact 120  
ccagagaaga gactggagtg ggtcgcatac gttagtgatg gtgggtggttc cacctactat 180  
ccagacattg taaagggccg attcaccatc tcccagagaca atgccaagaa caccctgtac 240  
cttcaaatga gcagtctgaa gtctgaggac acggccatgt atttctgtac aagacatgct 300  
tcctactata gctacgacca ttctatggac tactgggggc agggaacctc agtcaccgctc 360  
tcatca 366

<210> 174  
<211> 122  
<212> PRT  
<213> Ðj Êó

<400> 174

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

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

Pro Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val  
35 40 45

Ala Tyr Val Ser Asp Gly Gly Gly Ser Thr Tyr Tyr Pro Asp Ile Val  
50 55 60

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

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Phe Cys  
85 90 95

181708895-seql

Thr Arg His Ala Ser Tyr Tyr Ser Tyr Asp His Ser Met Asp Tyr Trp  
100 105 110

Gly Gln Gly Thr Ser Val Thr Val Ser Ser  
115 120

<210> 175  
<211> 357  
<212> DNA  
<213> Ðĭ Ėó

<400> 175  
cagatccagt tgggtgcagtc tgggggagac ttagtgaggc ctggaggggc cctgaaactc 60  
tcctgtgcag cctctggatt cactttcagt agctttggca tgtcttggat tcgccagact 120  
ccagacaaga ggctggagtg ggtcgcaacc attagtagtg ctggtagttt cacctactat 180  
ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaggaa caccctgtat 240  
ctacaaatga acagtctgaa gtctgaggac tcagccatgt attactgtgc aagacggggg 300  
tacgacgttg ggtgctttga ccactggggc cgaggcacca ctctcacagt ctctca 357

<210> 176  
<211> 119  
<212> PRT  
<213> Ðĭ Ėó

<400> 176

Gln Ile Gln Leu Val Gln Ser Gly Gly Asp Leu Val Arg Pro Gly Gly  
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe  
20 25 30

Gly Met Ser Trp Ile Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val  
35 40 45

Ala Thr Ile Ser Ser Ala Gly Ser Phe Thr Tyr Tyr Pro Asp Ser Val  
50 55 60

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

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Ser Ala Met Tyr Tyr Cys  
85 90 95

Ala Arg Arg Gly Tyr Asp Val Gly Cys Phe Asp His Trp Gly Arg Gly  
100 105 110

181708895-seql

Thr Thr Leu Thr Val Ser Ser  
115

<210> 177  
<211> 348  
<212> DNA  
<213> Ðĭ Êó

<400> 177  
gagggtgcacc tgggtggagtc tggggggaggc ttagtgcagc ctggagggtc cctgaaactc 60  
tcctgtgcag cctctggatt cactttcagt acctatggca tgtcttgggt tcgccagact 120  
ccagacaaga ggctggagtt ggtcgcgacc attaatacta atggtggtac cacctattat 180  
cgagacagtg tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac 240  
ctgcaaata gaagctctgaa gtctgatgac acagccatgt attactgtgc aagagactac 300  
ggggctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca 348

<210> 178  
<211> 116  
<212> PRT  
<213> Ðĭ Êó

<400> 178

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

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Leu Val  
35 40 45

Ala Thr Ile Asn Thr Asn Gly Gly Thr Thr Tyr Tyr Arg Asp Ser Val  
50 55 60

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

Leu Gln Met Ser Ser Leu Lys Ser Asp Asp Thr Ala Met Tyr Tyr Cys  
85 90 95

Ala Arg Asp Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val  
100 105 110

Thr Val Ser Ser  
115

181708895-seq1

<210> 179  
 <211> 345  
 <212> DNA  
 <213> Ðĵ Ěó

<400> 179  
 gatgtgcacc tgggtggagtc tggggggaggc ttagtgcagc ctggaggggtc cctgacagtc 60  
 tcctgcgag cctctggatt cactttcagt acctatggca tgtcttgggt tcgccagact 120  
 cgagacaaga ggctggagtt ggtcgcaacc ataaatacta atggtggttaa cacctattat 180  
 tcagacaatg tgaagggccg attcaccatt tccagagaca atgccaagaa caccctgtat 240  
 ttggaaatga gaggtctgag gtctggggac acagccatgt attactgtgc aagagactac 300  
 ggggctatgg actactgggg tcaaggaacc tcagtcaccg tctct 345

<210> 180  
 <211> 115  
 <212> PRT  
 <213> Ðĵ Ěó

<400> 180  
 Asp Val His Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Thr Val Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30  
 Gly Met Ser Trp Val Arg Gln Thr Arg Asp Lys Arg Leu Glu Leu Val  
 35 40 45  
 Ala Thr Ile Asn Thr Asn Gly Gly Asn Thr Tyr Tyr Ser Asp Asn Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Glu Met Arg Gly Leu Arg Ser Gly Asp Thr Ala Met Tyr Tyr Cys  
 85 90 95  
 Ala Arg Asp Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val  
 100 105 110  
 Thr Val Ser  
 115

<210> 181  
 <211> 348  
 <212> DNA  
 <213> Ðĵ Ěó

181708895-seq1

<400> 181  
gaggtgcagc tgcagcagcc tgggggaggc ttagtacagc ctggaggggc cctgacactc 60  
tcctgtgcaa cctctggatt cactttcagt agttatggca tgtcttgggt tcgccagact 120  
ccagccaaga ggctggagtt ggtcgcaacc attagtacta atgggtgccac cgccaattat 180  
ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaagag caccctgtac 240  
ctacaaatgc gcagtctgaa gtctgaggac acagccatgt attactgtgc aactgaaaag 300  
ggagctatgg gctactgggg tcaaggaacc tcagtcaccg tctcctca 348

<210> 182  
<211> 116  
<212> PRT  
<213> Ðĭ Ėó

<400> 182

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

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

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

Ala Thr Ile Ser Thr Asn Gly Ala Thr Ala Asn Tyr Pro Asp Ser Val  
50 55 60

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

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

Ala Thr Glu Lys Gly Ala Met Gly Tyr Trp Gly Gln Gly Thr Ser Val  
100 105 110

Thr Val Ser Ser  
115

<210> 183  
<211> 381  
<212> DNA  
<213> Ðĭ Ėó

<400> 183  
caagttactc taaaagagtc tggccctggg atattgaagc cctcacagac cctcagtctg 60  
acttgttctt tctctggggt ttactgacc acttctggta tgggtgtagg ctggattcgt 120

181708895-seq1

cagccttcag ggaaggggtct ggagtggctg gcacacattt ggtgggatga tgataagtac 180  
tataatccat ccctgaagag ccaggtcaca atctccaagg acacctccag aaaccaggta 240  
ttcctcaaga tcaccagtgt ggacactgca gatactgcca ctactactg tgctcgaaga 300  
actgagacta tgattacgac agtgctatat tactatgcta tggactactg gggtaagga 360  
acctcagtca ccgtctcctc a 381

<210> 184  
<211> 127  
<212> PRT  
<213> Ðĭ Ėó

<400> 184

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln  
1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Thr Thr Ser  
20 25 30

Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu  
35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser  
50 55 60

Leu Lys Ser Gln Val Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val  
65 70 75 80

Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala Arg Arg Thr Glu Thr Met Ile Thr Thr Val Leu Tyr Tyr Tyr  
100 105 110

Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser  
115 120 125

<210> 185  
<211> 378  
<212> DNA  
<213> Ðĭ Ėó

<400> 185

caagttactc taaaagagtc tggccctggg atattgaagc cctcacagac cctcagtctg 60  
acttgttctt tctctggatt ttactgagc acttctgggt tgggtgtagg ctggattcgt 120  
cagccttcag ggaaggggtct ggagtggctg gcacacattt ggtgggatga tgataagtac 180  
tataatccat cccttaagag acagatcaca atctccaagg attcctccag aaaccaggta 240



181708895-seq1

ttcctcaaga tcaccaatgt ggacactgca gatactgcca cttactactg tgctcgaagg 300  
 agggaagtta acttcggtat taactattac tattctatgg actactgggg tcaaggaacc 360  
 tcagtcaccg tctcctca 378

<210> 186  
 <211> 126  
 <212> PRT  
 <213> Ðí Êó

<400> 186

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln  
 1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser  
 20 25 30

Gly Leu Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu  
 35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser  
 50 55 60

Leu Lys Arg Gln Ile Thr Ile Ser Lys Asp Ser Ser Arg Asn Gln Val  
 65 70 75 80

Phe Leu Lys Ile Thr Asn Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Arg Arg Glu Val Asn Phe Gly Ile Asn Tyr Tyr Tyr Ser  
 100 105 110

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser  
 115 120 125

<210> 187  
 <211> 363  
 <212> DNA  
 <213> Ðí Êó

<400> 187

gaggtgaagc tgggtggagtc tggaggacgc ttggtacagc ctgggaattc tctgagactc 60  
 tcctgtgcaa cttctggatt cacccttcagt gattattaca tgagttgggt ccgccagact 120  
 ccaggaaggg cacttgagtg gttgagtttt attagaaatc gggctaattg ttacacaaca 180  
 gagtacagtg catctgtgaa gggtcgattc accatctcca gagataattc ccaaagcatc 240  
 ctctatcttc acatgagcac cctgagacct gaggacagtg ccacttatta ctgtgtaaga 300

181708895-seq1  
gatttcctatc actacgggta cttcgaatg tggggcgag ggaccacggt caccgtctcc 360  
tca 363

<210> 188  
<211> 121  
<212> PRT  
<213> Ðj Êó  
<400> 188

Glu Val Lys Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Asn  
1 5 10 15  
Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Tyr  
20 25 30  
Tyr Met Ser Trp Val Arg Gln Thr Pro Gly Arg Ala Leu Glu Trp Leu  
35 40 45  
Ser Phe Ile Arg Asn Arg Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala  
50 55 60  
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile  
65 70 75 80  
Leu Tyr Leu His Met Ser Thr Leu Arg Pro Glu Asp Ser Ala Thr Tyr  
85 90 95  
Tyr Cys Val Arg Asp Ser Tyr His Tyr Gly Tyr Phe Asp Val Trp Gly  
100 105 110  
Ala Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 189  
<211> 360  
<212> DNA  
<213> ÊÊ  
<400> 189  
caggtagacc tgaaggaatc cgggcctact ctggtgaaac ctaccacagac tctgactctg 60  
acttgacttt ttagcggcctt ctactgacc acatctggac tgggagtggc ttggatcaga 120  
cagcctcctg gaaaggccct ggagtggctg gctcacattt ggagcgacgg cgatactcgg 180  
tactatccag ccctgaaaaa cagactgact atcaccaagg acacatccaa aaaccaggtg 240  
gtcctgacaa tgactaatat ggaccccgctc gataccgcaa catactattg cgcccatatg 300  
aaggatgact ctctgtactt tgataactgg gggcagggaa ctctggtgac cgtgagcagc 360

181708895-seq1

<210> 190  
<211> 120  
<212> PRT  
<213> EE

<400> 190

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

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Thr Thr Ser  
20 25 30

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

Trp Leu Ala His Ile Trp Ser Asp Gly Asp Thr Arg Tyr Tyr Pro Ala  
50 55 60

Leu Lys Asn Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val  
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala His Met Lys Asp Asp Ser Leu Tyr Phe Asp Asn Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 191  
<211> 348  
<212> DNA  
<213> EE

<400> 191

gaggtgcagc tgctggaatc tgggggggga ctggtgcagc ctggaggaag cctgagactg	60
agttgtgccg caagtgggtt tacatttagc tcctacggaa tgagctgggt gaggcaggct	120
ccaggcaagg gactggagtg ggtctctgca atcagtacca acggagccac agcttactat	180
gccgactccg tgaagggccg gttcactatc tcaagagata acagcaagaa caccctgtat	240
ctgcagatga attctctgcg ggcagaagac acagccgtct actattgctg tactgagaaa	300
ggggcaatga gccactgggg acagggcaca ctggtgaccg tgagttcc	348

<210> 192  
<211> 116  
<212> PRT  
<213> EE

```

<210> 199
<211> 20
<212> DNA
<213> Artificial sequence

<400> 199
tttggrggga agatgaagac 20

<210> 200
<211> 21
<212> DNA
<213> Artificial sequence

<400> 200
ttaacactct cccctgttga a 21

<210> 201
<211> 21
<212> DNA
<213> Artificial sequence

<400> 201
ttaacactca ttcctgttga a 21

<210> 202
<211> 21
<212> DNA
<213> Artificial sequence

<400> 202
tggacaggga tccagagttc c 21

<210> 203
<211> 21
<212> DNA
<213> Artificial sequence

<400> 203
tggacagggc tccatagttc c 21

<210> 204
<211> 20
<212> DNA
<213> Artificial sequence

<400> 204
actcgtcctt ggtcaacgtg 20

<210> 205
<211> 9
<212> PRT
<213> Mus muscus

<400> 205

Gln His Ser Tyr Ser Trp Pro Trp Thr
1 5

```

181708895-seql

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr  
20 25 30

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln  
35 40 45

Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr  
50 55 60

Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg  
65 70 75 80

His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro  
85 90 95

Ile Val Lys Ser Phe Asn Arg Asn Glu Cys  
100 105

<210> 195  
<211> 315  
<212> DNA  
<213> Ðĭ Ėó

<400> 195  
ggtcagccca agtcactcc cactctcacc gtgtttccac cttcctctga ggagctcaag 60  
gaaaacaaag ccacactggg gtgtctgatt tccaactttt ccccgagtgg tgtgacagtg 120  
gcctggaagg caaatggtag acctatcacc caggggtgtgg acacttcaaa tcccacaaaa 180  
gagggcaaca agttcatggc cagcagcttc ctacatttga catcggacca gtggagatct 240  
cacaacagtt ttacctgtca agttacacat gaaggggaca ctgtggagaa gagtctgtct 300  
cctgcagaat gtctc 315

<210> 196  
<211> 105  
<212> PRT  
<213> Ðĭ Ėó

<400> 196

Gly Gln Pro Lys Ser Thr Pro Thr Leu Thr Val Phe Pro Pro Ser Ser  
1 5 10 15

Glu Glu Leu Lys Glu Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asn  
20 25 30

Phe Ser Pro Ser Gly Val Thr Val Ala Trp Lys Ala Asn Gly Thr Pro  
35 40 45

181708895-seq1

I l e Thr G l n G l y Val Asp Thr Ser Asn Pro Thr Lys Gl u G l y Asn Lys  
50 55 60

Phe Met Al a Ser Ser Phe Leu Hi s Leu Thr Ser Asp G l n Trp Arg Ser  
65 70 75 80

Hi s Asn Ser Phe Thr Cys G l n Val Thr Hi s Gl u G l y Asp Thr Val Gl u  
85 90 95

Lys Ser Leu Ser Pro Al a Gl u Cys Leu  
100 105

<210> 197  
<211> 975  
<212> DNA  
<213> Ðj Ěó

<400> 197  
gccaaaacaa cagcccatc ggtctatcca ctggcccctg tgtgtggaga tacaactggc 60  
tcctcggatga ctctaggatg cctgggtcaag ggttatttcc ctgagccagt gaccttgacc 120  
tggaactctg gatccctgtc cagcgggtgtg cacaccttcc cagctgtcct gcagtctgac 180  
ctctacactc tgagcagctc agtgactgtc cctccagca cctggcccag cgagaccgtc 240  
acctgcaacg ttgccacccc ggccagcagc accaagggtg acaagaaaat tgtgccagg 300  
gatttgtggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc 360  
ccccaaagc ccaaggatgt gctcaccatt actctgactc ctaaggtcac gtgtgttgtg 420  
gtagacatca gcaaggatga tcccagggtc cagttcagct ggtttgtaga tgatgtggag 480  
gtgcacacag ctgagcgc accccgggag gagcagttca acagcacttt ccgctcagtc 540  
agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc 600  
aacagtgcag ctttccctgc ccccatcgag aaaaccatct ccaaaaccaa aggcagaccg 660  
aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc 720  
agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtgg 780  
aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct 840  
tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc 900  
acctgctctg tgttacatga gggcctgcac aaccaccata ctgagaagag cctctccac 960  
tctcctggta aaggc 975

<210> 198  
<211> 325  
<212> PRT  
<213> Ðj Ěó

<400> 198

181708895-seql

Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly  
1 5 10 15  
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr  
20 25 30  
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser  
35 40 45  
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu  
50 55 60  
Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val  
65 70 75 80  
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys  
85 90 95  
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro  
100 105 110  
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu  
115 120 125  
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser  
130 135 140  
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu  
145 150 155 160  
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr  
165 170 175  
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn  
180 185 190  
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro  
195 200 205  
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln  
210 215 220  
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val  
225 230 235 240  
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val  
245 250 255

181708895-seq1

Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln  
260 265 270

Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn  
275 280 285

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val  
290 295 300

Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His  
305 310 315 320

Ser Pro Gly Lys Gly  
325

<210> 199  
<211> 20  
<212> DNA  
<213> °İ³ÉµÄÐðÁÐ

<400> 199  
tttggrrggga agatgaagac 20

<210> 200  
<211> 21  
<212> DNA  
<213> °İ³ÉµÄÐðÁÐ

<400> 200  
ttaacactct cccctgttga a 21

<210> 201  
<211> 21  
<212> DNA  
<213> °İ³ÉµÄÐðÁÐ

<400> 201  
ttaacactca ttcctgttga a 21

<210> 202  
<211> 21  
<212> DNA  
<213> °İ³ÉµÄÐðÁÐ

<400> 202  
tggacagggga tccagagttc c 21

<210> 203  
<211> 21  
<212> DNA  
<213> °İ³ÉµÄÐðÁÐ



181708895-seq1

<400> 203  
 tggacagggc tccatagttc c

21

<210> 204  
 <211> 20  
 <212> DNA  
 <213> °i³ÉµÄððÄð

<400> 204  
 actcgtcctt ggtcaacgtg  
 iiii

20