

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2017284157 B2**

(54) Title
Anti-CD47 monoclonal antibody and application thereof

(51) International Patent Classification(s)
C07K 16/30 (2006.01) **C12N 5/20** (2006.01)
A61K 39/395 (2006.01) **C12N 15/13** (2006.01)
A61P 35/00 (2006.01) **G01N 33/574** (2006.01)
A61P 35/02 (2006.01) **G01N 33/577** (2006.01)
C07K 14/705 (2006.01) **G01N 33/68** (2006.01)

(21) Application No: **2017284157** (22) Date of Filing: **2017.06.13**

(87) WIPO No: **WO17/215585**

(30) Priority Data

(31) Number	(32) Date	(33) Country
201610436519.3	2016.06.17	CN

(43) Publication Date: **2017.12.21**

(44) Accepted Journal Date: **2020.07.23**

(71) Applicant(s)
Changchun Genescience Pharmaceuticals Corp.

(72) Inventor(s)
Feng, Xiao;Jin, Lei;Xiao, Liang;Wang, Tao;Qin, Suofu

(74) Agent / Attorney
Griffith Hack, Level 29 Northpoint 100 Miller Street, Sydney, NSW, 2060, AU

(56) Related Art
WO 2016081423 A1
WO 2015191861 A1

(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 21 日 (21.12.2017)

WIPO | PCT

(10) 国际公布号
WO 2017/215585 A1

(51) 国际专利分类号:

C07K 16/30 (2006.01) **G01N 33/577** (2006.01)
C12N 15/13 (2006.01) **G01N 33/574** (2006.01)
C07K 14/705 (2006.01) **A61K 39/395** (2006.01)
C12N 5/20 (2006.01) **A61P 35/00** (2006.01)
G01N 33/68 (2006.01) **A61P 35/02** (2006.01)

(21) 国际申请号: PCT/CN2017/088013

(22) 国际申请日: 2017 年 6 月 13 日 (13.06.2017)

(25) 申请语言: 中文

(26) 公布语言: 中文

(30) 优先权:
201610436519.3 2016年6月17日 (17.06.2016) CN

(71) 申请人: 长春金赛药业股份有限公司 (CHANGCHUN GENESCENCE PHARMACEUTICALS CORP.) [CN/CN]; 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。

(72) 发明人: 冯晓(FENG, Xiao); 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。金磊(JIN, Lei); 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。肖亮(XIAO, Liang); 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。王涛(WANG, Tao); 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。秦锁富(QIN, Suofu); 中国吉林省长春市高新区越达路1718号, Jilin 130012 (CN)。

(74) 代理人: 北京集佳知识产权代理有限公司 (UNITALEN ATTORNEYS AT LAW); 中国北京

市朝阳区建国门外大街22号赛特广场7层, Beijing 100004 (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, JO, 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: ANTI-CD47 MONOCLONAL ANTIBODY AND APPLICATION THEREOF

(54) 发明名称: 抗CD47单克隆抗体及其应用

(57) Abstract: An anti-CD47 monoclonal antibody and an application thereof. The provided anti-CD47 monoclonal antibody can effectively inhibit tumor growth. Blocking human SIRP and human CD47 signals may enhance macrophage phagocytosis of tumor cells, prevent the tumor cells from escaping a tumor immune defense system, and have an anti-tumor function. Blocking an association between the CD47 on a tumor cell surface and SIRP on a macrophage surface may block a "do not eat me" signal from the tumor cells, promoting macrophage recognition and uptake of the tumor cells, and thereby facilitating tumor cells to be swallowed. The association of the CD47 on a tumor cell surface and SIRP on a macrophage surface is a common "do not eat me" signal. The anti-CD47 antibody, as a very promising target in a tumor immune system, will play a powerful and effective role in human cancer therapy.

(57) 摘要: 抗CD47单克隆抗体及其应用。提供的抗CD47单抗能有效抑制肿瘤生长, 阻断人SIRP与人CD47信号可以促进巨噬细胞对肿瘤细胞的吞噬作用, 阻止肿瘤细胞逃避肿瘤免疫的防御系统, 发挥抗肿瘤的作用。阻断肿瘤细胞表面CD47与巨噬细胞表面的SIRP的结合, 可以阻断肿瘤细胞的"别吃我"信号, 促进巨噬细胞对肿瘤细胞的识别摄取, 促进肿瘤细胞被吞噬。肿瘤细胞表面的CD47与巨噬细胞表面SIRP的结合是一种普遍的"别吃我"信号, 抗CD47抗体作为肿瘤免疫系统中的非常有前景的靶点, 在人类肿瘤治疗方面发挥强大而有效的作用。

WO 2017/215585 A1

Anti-CD47 monoclonal antibody and use thereof

The present application claims priority to Chinese patent application No. 201610436519.3 filed with the Chinese Patent Office (SIPO) on June 17, 2016, entitled “An anti-CD47 monoclonal antibody and use thereof”, which is incorporated herein by reference in its entirety.

Technical Field

The present invention relates to the technical field of antibody drugs, specifically, to an anti-CD47 monoclonal antibody and use thereof.

Background Art

CD47

CD47, also known as integrin-associated protein (IAP), was originally found from co-purification of human placenta and integrin $\alpha V\beta_3$ and co-immunoprecipitation of platelets and β_3 integrin. It is a transmembrane glycoprotein widely expressed on cell surface, and belongs to the immunoglobulin superfamily.

CD47 is a crucial marker on cell surface, with a molecular weight between 47 kD and 55 kD. It structurally includes an amino-terminal extracellular variable region, a transmembrane region composed of 3 to 5 highly hydrophobic transmembrane segments and a hydrophilic carboxy-terminal cytoplasmic tail. It interacts with a variety of ligands such as integrins, SIRP α (signal regulatory protein α), SIRP γ , and thrombospondin.

SIRP α

Signal regulatory protein α (SIRP α) is also a transmembrane protein expressed primarily on the surface of macrophages, dendritic cells and nerve cells. Its extracellular domain contains three immunoglobulins superfamily-like regions in which the N-terminal region mediates the binding to CD47 and its intracellular domain has a typical immunoreceptor tyrosine inhibitory sequence (ITIM). Upon binding to CD47, ITIM of SIRP α is phosphorylated, and a cascade reaction occurs, and the phagocytosis of macrophages is inhibited.

CD47/SIRPα is involved in the mechanism of tumor immune escape

In the innate immune system, CD47, as a marker of self, functions by transmitting an inhibitory “do not eat me” signal through binding with SIRPα expressed by myeloid cells such as macrophages, neutrophils, and dendritic cells. Thus, the broad expression of CD47 under physiological conditions is to protect healthy cells from being eliminated by the innate immune system. However, tumor cells effectively escape immune surveillance by overexpressing CD47.

In recent years, the CD47 and CD47-SIRPα signaling systems have received extensive attention. Among them, the most remarkable thing is that it is a potential drug target for cancer treatment. Studies have confirmed that CD47 expression is upregulated in most human cancers (eg, NHL, AML, breast cancer, colon cancer, glioblastoma, glioma, ovarian cancer, bladder cancer, and prostate cancer), and the elevated level of CD47 expression is associated with invasive diseases and poor survival. Weissman of the Stanford University systematically studied the expression level of CD47 in various solid tumors. The results indicated that all human solid tumor cells showed high expression of CD47, and the average expression level was about 3.3 times higher than that of normal cells. Moreover, they found that the level of CD47 mRNA in patients with solid tumors was negatively correlated with the prognostic index.

Further experiments on xenograft animal models of in situ immunodeficient mouse have found that administration of anti-CD47 monoclonal antibodies can inhibit the growth and metastasis of large tumors, and can cure small tumors. Willingham et al. also demonstrated the efficacy and safety of anti-CD47 monoclonal antibodies in an experiment with a mouse model of in situ breast cancer. This study not only confirmed that high expression of CD47 is a common mechanism for tumor cells to evade immune surveillance, but also provided valuable reference for the treatment of tumors by blocking CD47-SIRPα signaling pathway.

Therapeutic anti-CD47 antibody

CD47 is highly expressed in many types of tumors and acts as a “do not eat me” signal to inhibit phagocytosis, which means that targeting the CD47-SIRPα pathway can be used as a therapeutic method for many types of tumors.

Through in vitro and in vivo experiments, RAUH et al. demonstrated that anti-CD47 blocking monoclonal antibody can promote macrophage phagocytosis of tumor cells, inhibit

the formation of acute myeloid leukemia (AML) in mice, and eliminate AML that has been successfully transplanted in vivo. It can also perform targeted elimination of leukemia stem cells (LSC). Study of CHAO et al. on acute lymphoblastic leukemia has found that anti-CD47 monoclonal antibody combined with rituximab can not only eliminate tumors in the original transplant site, but also eliminate tumors in blood circulation and tumors that spread to the liver, spleen, lymph nodes, etc., thereby achieving the effects of long-term survival and inhibition of tumor recurrence, while the use of anti-CD47 monoclonal antibody or anti-CD20 monoclonal antibody alone can only inhibit the growth rate of NHL but cannot completely eliminate NHL.

In order to further confirm the effect of anti-CD47 monoclonal antibody on tumors, WILLINGHAM et al. establishes a xenograft tumor model using immunocompetent mouse. It was confirmed that anti-mouse and anti-human CD47 monoclonal antibodies both significantly inhibit tumor growth and anti-CD47 antibodies can eliminate a variety of solid tumors and inhibit tumor metastasis and recurrence. In addition, anti-CD47 monoclonal antibody also has an anti-tumor effect on cancer stem cells (CSC) and its differentiated subtypes, and can transform tumorigenic TAM into anti-tumor effector factors and enhance their phagocytosis. Inhibition of CD47 expression of mouse can also enhance the sensitivity of tumor cells to radiotherapy, while it has a protective effect on normal tissues, which may be associated with the induction of protective autophagy in host immune cells.

Treatment of tumors with anti-CD47 monoclonal antibodies is associated with a variety of mechanisms. First, anti-CD47 monoclonal antibodies block the binding of the CD47 on a tumor cell to SIRP α on a macrophage and thereby make tumor cells to be phagocytosed. Second, with respect to antibody-dependent and cell-mediated cytotoxicity and complement-dependent cytotoxicity, studies have found that anti-CD47 antibodies can induce cytotoxicity against head and neck tumor cells in which NK cells are involved. Third, tumor cells are eliminated by direct induction of apoptosis. Finally, studies on immunocompetent mice revealed that anti-CD47 monoclonal antibodies can activate CD8⁺ T cells, induce acquired T cell immune responses, and further kill tumor cells.

With the deepening of research on the tumor developing molecular mechanism, immunotherapy has gradually become another effective treatment means in addition to the

treatments such as surgery and chemical drugs. At present, the role of biotherapy in the treatment of tumors has been increasing year by year. Biotherapy has many advantages in preventing tumor recurrence, treating advanced cancer and its complications. Therefore, there is a need for antibodies and treatments that are capable of targeting CD47.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

Summary of the Invention

In view of the above, the present invention provides an anti-CD47 monoclonal antibody and use thereof. The anti-CD47 monoclonal antibody provided by the present invention can bind human CD47 and monkey CD47, and can block the binding between human SIRP and human CD47 in a dose-dependent manner, and thus promote macrophage phagocytosis of tumor cells. By blocking the binding signal between SIRP and human CD47, tumor cells are prevented from escaping a tumor immune defense system, thus achieving an anti-tumor effect.

A first aspect provides an isolated antigen comprising the amino acid sequence according to any one of SEQ ID NOs: 29 to 32.

A second aspect provides an anti-CD47 monoclonal antibody, comprising:

(I) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 1, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 8;

(II) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 2, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 9;

(III) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 3, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 10;

(IV) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 4, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 11;

(V) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 5, and a light chain variable region with an amino acid sequence represented by SEQ

ID NO: 12;

(VI) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 6, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 13; and

(VII) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 14.

Also disclosed is an anti-CD47 monoclonal antibody that has a heavy chain variable region and a light chain variable region:

(I) the amino acid sequence of the heavy chain variable region is represented by SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4 or SEQ ID NO: 5 or SEQ ID NO: 6 or SEQ ID NO: 7;

(II) the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 12 or SEQ ID NO: 13 or SEQ ID NO: 14;

(III) an amino acid sequence obtained by substituting or deleting one or more amino acids of the amino acid sequence represented by (I) or (II) or by adding one or more amino acids to the amino acid sequence represented by (I) or (II), and having the same or similar function as the amino acid sequence represented by (I) or (II); or

(IV) an amino acid sequence having at least 80% homology to the sequence represented by (I) or (II).

In some specific embodiments, in an amino acid sequence obtained by substituting or deleting one or more amino acids of the amino acid sequence of the anti-CD47 monoclonal antibody or by adding one or more amino acids to the amino acid sequence of the anti-CD47 monoclonal antibody, the more amino acids are two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one or thirty-two amino acids.

The substitution occurs in a hypervariable region;

the hypervariable regions of the heavy chain variable region are HVR-H1, HVR-H2, and HVR-H3;

in SEQ ID NO: 2, the hypervariable region HVR-H1 sequence is represented by SEQ

ID NO: 45; the HVR-H2 sequence is represented by SEQ ID NO: 46; and the HVR-H3 sequence is represented by SEQ ID NO: 47;

in SEQ ID NO: 5, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 48; the HVR-H2 sequence is represented by SEQ ID NO: 49; and the HVR-H3 sequence is represented by SEQ ID NO: 50; and

in SEQ ID NO: 6, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 51; the HVR-H2 sequence is represented by SEQ ID NO: 52; and the HVR-H3 sequence is represented by SEQ ID NO: 53;

the hypervariable regions of the light chain variable region are HVR-L1, HVR-L2, and HVR-L3;

in SEQ ID NO: 9, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 54; the HVR-L2 sequence is represented by SEQ ID NO: 55; and the HVR-L3 sequence is represented by SEQ ID NO: 56;

in SEQ ID NO: 12, the hypervariable region HVR-L1 sequence of is represented by SEQ ID NO: 57; the HVR-L2 sequence is represented by SEQ ID NO: 58; and the HVR-L3 sequence is represented by SEQ ID NO: 59; and

in SEQ ID NO: 13, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 60; the HVR-L2 sequence is represented by SEQ ID NO: 61; and the HVR-L3 sequence is represented by SEQ ID NO: 62.

The amino acid sequence of the heavy chain variable region is represented by any one of SEQ ID NOs: 1 to 7;

The amino acid sequence of the light chain variable region is represented by any one of SEQ ID NOs: 8 to 14.

In some specific embodiments, the anti-CD47 monoclonal antibody comprises:

(i) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 8;

(ii) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 9;

(iii) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence

represented by SEQ ID NO: 10;

(iv) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 11;

(V) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 12;

(VI) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 13; and

(VII) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 14.

The anti-CD47 monoclonal antibody provided by the present invention has a heavy chain type of IgG1, IgG3 or IgM; and its light chain type is κ .

A third aspect of the present invention provides a nucleotide sequence or sequences encoding the anti-CD47 monoclonal antibody according to the second aspect.

In some specific embodiments, the nucleotide sequence comprises

(I) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(II) a sequence complementary with the nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a sequence complementary with the nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(III) a sequence which encodes the same protein as the nucleotide sequence of (I) or (II) but differs from the nucleotide sequence of (I) or (II) due to the degeneracy of the genetic code; or

(IV) a sequence having at least 80% homology to the sequence of (I) or (II) or (III).

In some specific embodiments, the nucleotide has a nucleotide sequence obtained by substituting or deleting one or more nucleotides of the nucleotide sequence represented by (I) or (II) or (III) or (IV) or adding one or more nucleotides to the nucleotide sequence

represented by (I) or (II) or (III) or (IV), and having the same or similar function as the nucleotide sequence represented by (I) or (II) or (III) or (IV).

In some specific embodiments, the nucleotide sequence has a nucleotide sequence obtained by substituting or deleting one or more nucleotides of the nucleotide sequence represented by (I) or (II) or (III) or (IV) or adding one or more nucleotides to the nucleotide sequence represented by (I) or (II) or (III) or (IV), and the more nucleotides are two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one or thirty-two nucleotides.

A fourth aspect of the present invention provides an expression vector or vectors comprising the nucleotide sequence or sequences according to the third aspect.

A fifth aspect of the present invention provides a host cell transformed with the expression vector or vectors according to the fourth aspect.

A sixth aspect of the present invention provides a hybridoma cell strain, which produces the anti-CD47 monoclonal antibody according to the second aspect.

A seventh aspect provides a method for preparing the anti-CD47 monoclonal antibody according to the second aspect, wherein the method comprises:

step 1: after immunizing a mouse with the antigen according to the first aspect, obtaining spleen cells of the mouse;

step 2: fusing the spleen cells with myeloma cells, screening for a hybridoma cell strain capable of binding to CD47, and culturing the obtained cell strain in vitro to obtain the anti-CD47 monoclonal antibody.

An eighth aspect provides a combination, which is prepared by marking the anti-CD47 monoclonal antibody according to the second aspect with a chemical marker or a biomarker.

The chemical marker is an isotope, an immunotoxin, and/or a chemical drug.

The biomarker is biotin, avidin or an enzyme label.

A ninth aspect of the present invention provides a conjugate, which is prepared by coupling the anti-CD47 monoclonal antibody according to the second aspect or the combination according to the eighth aspect to a solid medium or a semi-solid medium.

A tenth aspect provides use of the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect and/or the conjugate according

to the ninth aspect in the preparation of a product for detecting expression of CD47.

An eleventh aspect of the present invention provides a kit comprising the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect and/or the conjugate according to the ninth aspect.

Also disclosed is a method for diagnosing disease comprises detecting the expression of CD47 using the kit of the present disclosure, and determining whether or not a disease is developed according to the expression level of CD47.

The disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

A twelfth aspect provides use of the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect and/or the conjugate according to the ninth aspect in the preparation of a formulation for blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells

In an embodiment of the present invention, the tumor cells are human peripheral blood leukemia T cells.

A thirteenth aspect provides a method of blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells, comprising administering the anti-CD47 monoclonal antibody according to the second aspect, the combination according to according to the eighth aspect, and/or the conjugate according to according to the ninth aspect.

A fourteenth aspect provides use of the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect and/or the conjugate according to the ninth aspect in the preparation of a medicament for preventing and treating disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

A fifteenth aspect of the present invention provides a medicament comprising the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect and/or the conjugate according to the ninth aspect.

A sixteenth aspect provides a method for preventing or treating disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma, comprising administering the anti-CD47 monoclonal antibody according to the second aspect, the combination according to the eighth aspect, the conjugate according to the ninth aspect, and/or the medicament according to the fifteenth aspect.

The anti-CD47 monoclonal antibody disclosed herein can effectively inhibit tumor growth. Blocking the signal between human SIRP and human CD47 may enhance macrophage phagocytosis of tumor cells, prevent the tumor cells from escaping a tumor immune defense system, and achieve an anti-tumor function. Blocking the binding of the CD47 on a tumor cell surface to SIRP on a macrophage surface may block a “do not eat me” signal from the tumor cell, promote the tumor cell recognition and uptake of macrophages, and thereby facilitating tumor cells to be phagocytosed. The binding of the CD47 on a tumor cell surface to SIRP on a macrophage surface is a common “do not eat me” signal. The anti-CD47 antibody can be used as a very promising target in the tumor immune system, and play a powerful and effective role in human cancer therapy.

The present disclosure includes the following items 1 to 21:

1. An isolated antigen comprising the amino acid sequence according to any one of SEQ ID NOs: 29 to 32.
2. An anti-CD47 monoclonal antibody that specifically binds to the antigen according to item 1, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein:

(I) the amino acid sequence of the heavy chain variable region is represented by SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4 or SEQ ID NO: 5 or SEQ ID NO: 6 or SEQ ID NO: 7;

(II) the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 12 or SEQ ID NO: 13 or SEQ ID NO: 14;

(III) hypervariable regions of the heavy chain variable region are HVR-H1, HVR-H2, and HVR-H3;

wherein in SEQ ID NO: 2, the hypervariable region HVR-H1 sequence is represented by

SEQ ID NO: 45; the HVR-H2 sequence is represented by SEQ ID NO: 46; and the HVR-H3 sequence is represented by SEQ ID NO: 47;

wherein in SEQ ID NO: 5, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 45; the HVR-H2 sequence is represented by SEQ ID NO: 46; and the HVR-H3 sequence is represented by SEQ ID NO: 47; and

wherein in SEQ ID NO: 6, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 51; the HVR-H2 sequence is represented by SEQ ID NO: 52; and the HVR-H3 sequence is represented by SEQ ID NO: 53; and

hypervariable regions of the light chain variable region are HVR-L1, HVR-L2, and HVR-L3;

wherein in SEQ ID NO: 9, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 60; the HVR-L2 sequence is represented by SEQ ID NO: 55; and the HVR-L3 sequence is represented by SEQ ID NO: 56;

wherein in SEQ ID NO: 12, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 54; and the HVR-L2 sequence is represented by SEQ ID NO: 55; and

in SEQ ID NO: 13, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 60; the HVR-L2 sequence is represented by SEQ ID NO: 61; and the HVR-L3 sequence is represented by SEQ ID NO: 56; or

(IV) the antibody comprises an amino acid sequence having at least 80% homology to the sequence represented by (I) or (II), and wherein the antibody specifically binds to CD47.

3. The anti-CD47 monoclonal antibody according to item 2, wherein the antibody comprises:

(I) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 8;

(II) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 9;

(III) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 10;

(IV) a heavy chain variable region with an amino acid sequence represented by SEQ ID

NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 11;

(V) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 12;

(VI) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 13; and

(VII) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 14.

4. The anti-CD47 monoclonal antibody according to item 2 or item 3, wherein the heavy chain type is IgG1, IgG3 or IgM; and the light chain type is κ .

5. A nucleotide sequence or sequences encoding the anti-CD47 monoclonal antibody according to any one of items 2 to 4.

6. The nucleotide sequence or sequences according to claim 5, comprising:

(I) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(II) a sequence complementary with the nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a sequence complementary with the nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(III) a nucleotide sequence which encodes the same protein as the nucleotide sequence of (I) or (II) but differs from the nucleotide sequence of (I) or (II) due to the degeneracy of the genetic code; or

(IV) a sequence having at least 80% homology to the sequence of (I) or (II) or (III).

7. An expression vector or vectors comprising the nucleotide sequence or sequences according to claim 5 or claim 6.

8. A host cell transformed with the expression vector or vectors according to item 7

9. A hybridoma cell strain, which produces the anti-CD47 monoclonal antibody according to any one of items 2 to 4.

10. A method for preparing the anti-CD47 monoclonal antibody according to any one of items 2 to 4, wherein the method comprises:

step 1: after immunizing a mouse with the antigen according to item 1, obtaining spleen cells of the mouse;

step 2: fusing the spleen cells with myeloma cells, screening for a hybridoma cell strain capable of binding to CD47, and culturing the cell strain *in vitro* to obtain the anti-CD47 monoclonal antibody.

11. A combination, which is prepared by marking the anti-CD47 monoclonal antibody according to any one of items 2 to 4 with a chemical or biological marker.

12. The combination according to item 11, wherein the chemical marker is an isotope, an immunotoxin and/or a chemical drug; and the biological marker is biotin, avidin or an enzyme label.

13. A conjugate, which is prepared by coupling the anti-CD47 monoclonal antibody according to any one of items 2 to 4 or the combination according to item 11 or item 12 to a solid medium or a semi-solid medium.

14. Use of the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, and/or the conjugate according to item 13 in the preparation of a product for detecting expression of CD47.

15. A kit comprising the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, and/or the conjugate according to item 13.

16. Use of the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to claim 11 or claim 12, and/or the conjugate according to claim 13 in the preparation of a formulation for blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells.

17. A method of blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells, comprising administering the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, and/or the conjugate according to item 13.

18. The use according to item 16, of the method according to item 17, wherein the tumor

cells are human peripheral blood leukemia T cells.

19. Use of the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, and/or the conjugate according to item 13 in the preparation of a medicament for preventing or treating a disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

20. A medicament comprising the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, and/or the conjugate according to item 13.

21. A method for preventing or treating a disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma, comprising administering the anti-CD47 monoclonal antibody according to any one of items 2 to 4, the combination according to item 11 or item 12, the conjugate according to item 13, and/or the medicament of item 20.

Brief Description of the Drawings

Figure 1 shows SDS-PAGE electrophoresis detection of purified human and monkey

CD47: lane M: protein molecular weight marker; Figure 1-A: lane 1: human CD47-linker peptide-hIgG1Fc; lane 2: human CD47-linker peptide-His; Figure 1-B, lane 1: mouse CD47-linker peptide-hIgG1Fc; lane 2: mouse CD47-linker peptide-His.

Figure 2 shows SDS-PAGE electrophoresis detection of positive antibody (PAB): lane M: protein molecular weight marker; lane 1: non-reducing PAB; lane 2: reducing PAB.

Figure 3 shows non-reducing SDS-PAGE electrophoresis detection of a purified candidate antibody: lane M: protein molecular weight marker; lane 1: 059-1.11.1 purified antibody; lane 2: 059-1.20.1 purified antibody; lane 3: 059-1.30.1 purified antibody; lane 4: 059-1.43.1 purified antibody; lane 5: 059-1.51.2 purified antibody; lane 6: 059-1.82.1 purified antibody; lane 7: 059-1.100.5 purified antibody.

Figure 4 shows electrophoresis of total RNA. Lane M: DL2000 molecular weight marker. Lanes 1-7 are total RNA electrophoresis bands of 059-1.11.1, 059-1.20.1, 059-1.30.1, 059-1.43.1, 059-1.51.2, 059-1.82.1, 059-1.100.5, respectively.

Figure 5 shows agarose electrophoresis detection results of heavy chain variable region and light chain variable region of PCR amplified candidate antibodies.

Figure 6 is a graph showing the results of blocking human CD47 and SIRP α by antibodies 059-1.20.1, 059-1.30.1, 059-1.43.1, and 059-1.82.1.

Figure 7 shows FACS assays for binding of antibodies 059-1.20.1, 059-1.30.1, 059-1.43.1, 059-1.82.1 to CD47 on jurkat cell surface.

Figure 8 shows that antibodies 059-1.20.1, 059-1.30.1, 059-1.43.1, 059-1.82.1 promote mouse peritoneal primary macrophages's phagocytosis of Jurkat cells.

Specific Modes for Carrying Out the Embodiments

The present invention provides an anti-CD47 monoclonal antibody and use thereof, which can be achieved by a person skilled in the art by learning from the contents herein and appropriately improving the process parameters. It is to be understood that all such alternatives and modifications are obvious to a person skilled in the art and are considered to be included in the present invention. The method and the use according to the present invention have been described by the preferred embodiments, and it will be apparent to a person skilled in the art that the method and the use according to the present invention may be modified or appropriately altered and combined without departing from the scope, spirit and

range of the present invention to achieve and apply the present invention.

“Antibody” refers to a protein composed of one or more polypeptides, which is capable of specifically binding an antigen. One form of antibody constitutes the basic structural unit of an antibody. This form is a tetramer consisting of two identical pairs of antibody chains, each pair having a light chain and a heavy chain. In each pair of antibody chains, the variable regions of the light chain and heavy chain are joined together to bind the antigen, while the constant region is responsible for the effector function of the antibody.

The “variable region” of an antibody heavy chain or light chain is the N-terminal mature region of the chain. Currently known types of antibodies include κ and λ light chains, as well as α , γ (IgG1, IgG2, IgG3, IgG4), δ , ϵ and μ heavy chains or equivalents thereof belonging to other types. The full length immunoglobulin “light chain” (approximately 25 kDa or approximately 214 amino acids) comprises a variable region formed by approximately 110 amino acids at the NH₂-terminal, and a κ or λ constant region at the COOH-terminal. A full length immunoglobulin “heavy chain” (approximately 50 kDa or approximately 446 amino acids) also contains a variable region (approximately 116 amino acids) and one of the heavy chain constant regions, such as λ (approximately 330 amino acids).

“Antibody” includes any isotype of antibodies or immunoglobulins, or antibody fragments that retain the ability of specifically binding to an antigen, including but not limited to Fab, Fv, scFv and Fd fragments, chimeric antibodies, humanized antibodies, single chain antibodies, and a fusion protein comprising an antigen binding portion of an antibody and a non-antibody protein. The antibody can be labeled and detected, for example, by a radioisotope, an enzyme capable of producing a detectable substance, a fluorescent protein, biotin, or the like. Antibodies can also bind to a solid support, including but not limited to polystyrene plates or beads, and the like.

The present invention provides an anti-CD47 monoclonal antibody having a heavy chain variable region and a light chain variable region:

(I) the amino acid sequence of the heavy chain variable region is represented by SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4 or SEQ ID NO: 5 or SEQ ID NO: 6 or SEQ ID NO: 7;

(II) the amino acid sequence of the light chain variable region is represented by SEQ ID

NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 12 or SEQ ID NO: 13 or SEQ ID NO: 14;

(III) an amino acid sequence obtained by substituting or deleting one or more amino acids of the amino acid sequence represented by (I) or (II) or by adding one or more amino acids to the amino acid sequence represented by (I) or (II), and having the same or similar function as the amino acid sequence represented by (I) or (II); or

(IV) an amino acid sequence having at least 80% homology to the sequence represented by (I) or (II).

In some specific embodiments of the present invention, in said amino acid sequence obtained by substituting or deleting one or more amino acids of the amino acid sequence of the anti-CD47 monoclonal antibody or by adding one or more amino acids to the amino acid sequence of the anti-CD47 monoclonal antibody, the more amino acids are two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one or thirty-two amino acids.

The substitution occurs in a hypervariable region;

the hypervariable regions of the heavy chain variable region are HVR-H1, HVR-H2, and HVR-H3;

in SEQ ID NO: 2, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 45; the HVR-H2 sequence is represented by SEQ ID NO: 46; and the HVR-H3 sequence is represented by SEQ ID NO: 47;

in SEQ ID NO: 5, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 48; the HVR-H2 sequence is represented by SEQ ID NO: 49; and the HVR-H3 sequence is represented by SEQ ID NO: 50; and

in SEQ ID NO: 6, the hypervariable region HVR-H1 sequence is represented by SEQ ID NO: 51; the HVR-H2 sequence is represented by SEQ ID NO: 52; and the HVR-H3 sequence is represented by SEQ ID NO: 53;

the hypervariable regions of the light chain variable region are HVR-L1, HVR-L2, and HVR-L3;

in SEQ ID NO: 9, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 54; the HVR-L2 sequence is represented by SEQ ID NO: 55; and the HVR-L3 sequence is represented by SEQ ID NO: 56;

in SEQ ID NO: 12, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 57; the HVR-L2 sequence is represented by SEQ ID NO: 58; and the HVR-L3 sequence is represented by SEQ ID NO: 59; and

in SEQ ID NO: 13, the hypervariable region HVR-L1 sequence is represented by SEQ ID NO: 60; the HVR-L2 sequence is represented by SEQ ID NO: 61; and the HVR-L3 sequence is represented by SEQ ID NO: 62.

In some specific embodiments of the present invention, the anti-CD47 monoclonal antibody comprises:

(i) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 8;

(ii) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 9;

(iii) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 10;

(iv) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 11;

(V) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 12;

(VI) a heavy chain variable region with an amino acid sequence represented by SEQ ID NOs: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 13;

(VII) a heavy chain variable region with an amino acid sequence represented by SEQ ID

NOS: 1, 2, 3, 4, 5, 6, or 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 14.

The anti-CD47 monoclonal antibody provided by the present invention has a heavy chain type of IgG1, IgG3 or IgM; and its light chain type is κ .

5 Specifically, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid
25 sequence of the light chain variable region is represented by SEQ ID NO: 8.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the
30 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid

sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

5 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 10.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid
25 sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the
30 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid

sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 10.

5 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid
25 sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the
30 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid

sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

5 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid
25 sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the
30 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid

sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 13.

5 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 13.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid
25 sequence of the light chain variable region is represented by SEQ ID NO: 14.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

Alternatively, the amino acid sequence of the heavy chain variable region of the
30 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid

sequence of the light chain variable region is represented by SEQ ID NO: 14.

In an embodiment of the present invention, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 1, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 8.

5 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 2, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 9.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 3, and the amino acid
10 sequence of the light chain variable region is represented by SEQ ID NO: 10.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 4, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 11.

Alternatively, the amino acid sequence of the heavy chain variable region of the
15 anti-CD47 monoclonal antibody is represented by SEQ ID NO: 5, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 12.

Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 6, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 13.

20 Alternatively, the amino acid sequence of the heavy chain variable region of the anti-CD47 monoclonal antibody is represented by SEQ ID NO: 7, and the amino acid sequence of the light chain variable region is represented by SEQ ID NO: 14.

The heavy chain constant region of the antibody 059-1.82.1 of the present invention is mouse IgG3, the light chain constant region is the constant region of the mouse κ chain, and
25 059-1.30.1, 059-1.43.1, and 059-1.20.1 are all mouse IgG1, the light chain constant region is the constant region of the mouse κ chain; the heavy chain constant regions of 059-1.11.1, 059-1.51.2, and 059-1.100.5 are mouse IgM. The amino acid sequence of the heavy chain variable region is one of SEQ ID NOs: 1 to 7, and the amino acid sequence of the light chain variable region is one of SEQ ID NOs: 8 to 14.

30 The anti-CD47 monoclonal antibody provided by the present invention is capable of

binding human CD47 and monkey CD47; in certain embodiments, the affinity between the antibody and its target is characterized by K_a , K_d (dissociation constant), and K_D (equilibrium dissociation constant). The K_D value of the antibody provided by the present invention is not higher than 30 nM.

5 The anti-CD47 monoclonal antibody provided by the present invention can block the binding between human SIRP and human CD47 in a dose-dependent manner; the blocking effect thereof is represented by an EC_{50} value, and the EC_{50} value of the antibody provided by the present invention is not less than 850 nM.

10 The anti-CD47 monoclonal antibody provided by the present invention is capable of binding to CD47 on cell surface; the detection of this effect is carried out by the FACS method, and the results of FACS are represented by MFI (fluorescence intensity), and the MFI value of the binding between the antibody provided by the present invention and the CD47 on cell surface is not less than 9547, and can be up to 18533.

15 The anti-CD47 monoclonal antibody provided by the present invention can promote macrophage phagocytosis of tumor cells, and the effect is measured by fluorescence imaging, and the result is expressed by the phagocytic index. The phagocytic index against jurkat cells of the antibody provided by the present invention can reach 79.

20 The anti-CD47 monoclonal antibody provided by the present invention can also induce apoptosis of tumor cells, and the effect is expressed by the cell apoptosis rate detected using a flow cytometry, and the results show that the antibody provided by the present invention can induce apoptosis of jurkat cells, and the apoptosis rate can reach 48%.

25 Jurkat cells belong to the acute T cell leukemia cell line and are one of a variety of malignant tumor cells. Like other malignant tumors, CD47 on the jurkat cell surface has a high expression level. In the present invention, it is proved by the experiment on jurkat cells that the CD47 monoclonal antibody provided by the present invention can prevent the tumor cells from escaping the tumor immune defense system by blocking the binding signal between SIRP and human CD47, and thereby achieving an anti-tumor effect.

30 In the monoclonal antibodies provided by the present invention, the monoclonal antibodies provided by the present invention with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 2 and the amino acid sequence of the light

chain variable region as represented by SEQ ID NO: 9, the monoclonal antibodies with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 3 and the amino acid sequence of the light chain variable region as represented by SEQ ID NO: 10, the monoclonal antibodies with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 4 and the amino acid sequence of the light chain variable region as represented by SEQ ID NO: 11, and the monoclonal antibodies with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 6 and the amino acid sequence of the light chain variable region as represented by SEQ ID NO: 13, have a good affinity for both human CD47 and monkey CD47, can block the binding between CD47 and SIRP α , and can bind to CD47 on cell surface, promote macrophage phagocytosis of tumor cells, and induce apoptosis of tumor cells.

Wherein the monoclonal antibody with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 4 and the amino acid sequence of the light chain variable region as represented by SEQ ID NO: 11, has the best effect of blocking the binding between CD47 and SIRP α . This monoclonal antibody also has binding to CD47 on tumor cell surface, and the best effect of inducing macrophage phagocytosis of tumor cells. However, for inducing tumor cell apoptosis, the monoclonal antibody with the amino acid sequence of the heavy chain variable region as represented by SEQ ID NO: 3 and the amino acid sequence of the light chain variable region as represented by SEQ ID NO: 10 shows superior effect.

The present invention also provides a nucleotide sequence encoding the anti-CD47 monoclonal antibody.

In some embodiments of the present invention, the nucleotide sequence comprises:

(I) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(II) a sequence complementary with the nucleotide sequence of the heavy chain variable region as represented by SEQ ID NOs: 15 to 21; a sequence complementary with the nucleotide sequence of the light chain variable region as represented by SEQ ID NOs: 22 to 28; or

(III) a sequence which encodes the same protein as the nucleotide sequence of (I) or (II) but differs from the nucleotide sequence of (I) or (II) due to the degeneracy of the genetic code; or

(IV) a sequence having at least 80% homology to the sequence of (I) or (II) or (III).

5 In some specific embodiments of the present invention, the nucleotide sequence has a nucleotide sequence obtained by substituting or deleting one or more nucleotides of the nucleotide sequence represented by (I) or (II) or (III) or (IV) or by adding one or more nucleotides to the nucleotide sequence represented by (I) or (II) or (III) or (IV), and having the same or similar function as the nucleotide sequence represented by (I) or (II) or (III) or
10 (IV).

In some specific embodiments of the present invention, the nucleotide sequence has a nucleotide sequence obtained by substituting or deleting one or more nucleotides of the nucleotide sequence represented by (I) or (II) or (III) or (IV) or by adding one or more nucleotides to the nucleotide sequence represented by (I) or (II) or (III) or (IV), and the more
15 nucleotides are two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one or thirty-two nucleotides.

The present invention also provides an expression vector comprising a nucleotide
20 sequence that provide a heavy chain variable region and/or a light chain variable region of the anti-CD47 monoclonal antibody provided by the present invention.

The present invention also provides a host cell transformed with the expression vector of the present invention.

The present invention also provides an antigen having the amino acid sequence
25 represented by any one of SEQ ID NOs: 29 to 32.

The present invention also provides a hybridoma cell strain which produces the anti-CD47 monoclonal antibody of the present invention.

The preparation method of the anti-CD47 monoclonal antibody provided by the present invention comprises:

30 step 1: after immunizing a mouse with the antigen provided by the present invention,

obtaining spleen cells of the mouse; and

step 2: fusing the spleen cells with myeloma cells, screening for a hybridoma cell strain capable of binding to CD47, and culturing the cell strain in vitro to obtain an anti-CD47 monoclonal antibody.

5 In the present invention, the amino acid sequence of the antigen in step 1 is represented by SEQ ID NO: 29 or 31.

In the present invention, the antigen is mixed with an adjuvant to immunize mice.

The volume ratio of the antigen to the adjuvant is 1:1.

Specifically, the immunization is performed as follows: two weeks after a first
10 immunization, a second immunization is performed, and 3 days later, a booster immunization is performed for the mice having a serum titer greater than 1:200,000.

The doses for the first immunization, the second immunization, and the booster immunization are all 10 µg in terms of antigen mass.

Immunization is performed by two-point injection.

15 The adjuvant for the first immunization is Freund's complete adjuvant, and the adjuvant for the second immunization and the booster immunization is Freund's incomplete adjuvant.

The mice used for immunization are BALB/C mice.

The myeloma cell is P3X63Ag8.653.

The fusion is performed at a ratio of spleen cells to myeloma cells of 5:1.

20 A HAT medium is used for screening.

Binding to CD47 specifically means the ability of binding to CD47 protein and the ability of binding to cells that express CD47 protein on the surface.

After identification, The CD47 monoclonal antibody prepared by the method of the present invention has a heavy chain type of IgG3, IgM or IgG1, and a light chain type of κ.

25 A combination made by marking the anti-CD47 monoclonal antibody of the present invention with a chemical marker or a biomarker.

The chemical marker is an isotope, an immunotoxin, and/or a chemical drug.

The biomarker is biotin, avidin or an enzyme label.

The enzyme label is preferably horseradish peroxidase or alkaline phosphatase.

30 The immunotoxin is preferably aflatoxin, diphtheria toxin, Pseudomonas aeruginosa

exotoxin, ricin, abrin, mistletoe lectin, modeccin, PAP, saporin, gelonin or luffin.

The present invention also provides a conjugate prepared by coupling the anti-CD47 monoclonal antibody or a combination thereof to a solid medium or a semi-solid medium.

5 The solid medium or non-solid medium is selected from colloidal gold, polystyrene plates or beads.

Use of the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the present invention in the preparation of a product for detecting the expression of CD47.

10 Experiments have shown that the CD47 monoclonal antibody provided by the present invention can bind to CD47 protein, and can also bind to cells expressing CD47 on the surface. Therefore, the CD47 monoclonal antibody provided by the present invention can be used for the detection of CD47 protein or cells expressing CD47 on the surface. Moreover, due to the high expression of the tumor cell surface marker CD47, the antibody provided by the present invention is capable of preparing a kit for the detection of the tumor surface marker CD47, wherein, the detection of the CD47 protein is carried out by a ELISA method, and the detection of cells expressing CD47 on the surface is carried out by a FACS method.

15 The present invention also provides a kit comprising the anti-CD47 monoclonal antibody, the combination and/or the conjugate.

The kit for detecting CD47 protein further includes a coating buffer, a washing solution, a blocking solution, and/or a color developing solution.

20 The coating buffer is a carbonate buffer.

The washing solution includes PBS, Tween, sodium chloride, potassium chloride, disodium hydrogen phosphate, and dipotassium hydrogen phosphate.

The blocking solution includes PBS and BSA.

25 The color developing solution includes a TMB solution, a substrate buffer, and a stop solution.

The substrate buffer includes citric acid and disodium hydrogen phosphate.

The stop solution is an aqueous hydrogen peroxide solution.

The kit for detecting cells expressing CD47 on the surface also include PBS, goat-anti-mouse IgG Fc and TITC secondary antibody.

30 Use of the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the

present invention in the preparation of a formulation for blocking the binding of CD47 to SIRP α .

The cells expressing CD47 on the surface are tumor cells.

5 The tumor cells are selected from leukemia cells, lymphoma cells, breast cancer cells, lung cancer cells, gastric cancer cells, intestinal cancer cells, esophageal cancer cells, ovarian cancer cells, cervical cancer cells, kidney cancer cells, bladder cancer cells, pancreatic cancer cells, glioma cells and / or melanoma cells.

A method for diagnosing disease comprises detecting CD47 expression using the kit provided by the present invention, and determining whether or not a disease is developed
10 according to the expression level of CD47.

The disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

Use of the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the
15 present invention in the preparation of a formulation for blocking the binding of CD47 to SIRP α .

The anti-CD47 monoclonal antibody of the present invention blocks the binding of CD47 to SIRP α with an EC50 value of 850 nM to 2340 nM.

Use of the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the
20 present invention in the preparation of a formulation for increasing macrophage phagocytic index of tumor cells.

The dose of the anti-CD47 monoclonal antibody of the present invention for increasing macrophage phagocytic index of tumor cells is 10 μ g/mL in terms of antibody concentration.

In the embodiment of the present invention, the tumor cells are human peripheral blood
25 leukemia T cells.

Use of the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the present invention in the preparation of a formulation for promoting apoptosis of tumor cells.

In an embodiment of the present invention, the tumor cells are human peripheral blood leukemia T cells.

30 The dose of the anti-CD47 monoclonal antibody of the present invention for promoting

apoptosis of tumor cells is 10 µg/mL.

Use of the anti-CD47 monoclonal antibody, combination and/or conjugate thereof in the preparation of a medicament for preventing and treating tumors.

5 The disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

The invention also provides a medicament comprising the anti-CD47 monoclonal antibody, the combination and/or the conjugate of the present invention.

10 A method for preventing and treating disease comprises administering the medicament of the present invention. The disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

15 The medicament provided by the present invention can promote tumor cell recognition and uptake of macrophages by blocking a “do not eat me” signal from the tumor cells, thereby facilitating tumor cells to be phagocytosed.

The dosage form of the medicament provided by the present invention is an injection solution or a powder injection.

The concentration of the antibody in the injection solution is 10 µg/mL.

20 The anti-CD47 monoclonal antibody provided by the present invention can effectively inhibit tumor growth. Blocking the signal between human SIRP and human CD47 may enhance macrophage phagocytosis of tumor cells, prevent the tumor cells from escaping the tumor immune defense system, and have an anti-tumor function. Blocking the binding of the CD47 on a tumor cell surface to SIRP on a macrophage surface may block a “do not eat me” signal from the tumor cells, promote the tumor cell recognition and uptake of macrophages, and thereby facilitating tumor cells to be phagocytosed. The binding between the CD47 on
25 tumor cell surface and SIRP on macrophage surface is a common “do not eat me” signal. The anti-CD47 antibody can be used as a very promising target in the tumor immune system, and play a powerful and effective role in human cancer therapy.

30 The instruments used in the present invention are all commercially available and can be purchased in the market.

The present invention is further illustrated below in combination with the Examples.

Example 1: Preparation of antigenic protein and positive control antibody

1.1 Synthesis of antigen gene and construction of expression vector:

The amino acid sequence of the extracellular region of the human-derived and monkey-derived CD47 protein was fused with the linker peptide-hIgG1Fc or the linker peptide-7his amino acid sequence, and the amino acid sequence was designed as represented by SEQ ID NOs: 29, 30, 31, and 32.

The amino acid sequences corresponding to the human and monkey CD47 protein extracellular region fusion protein (CD47ECD-linker peptide-hIgG1Fc or CD47ECD-linker peptide-7his) designed above were codon-optimized, for example, SEQ ID NOs: 33, 34, 35, and 36 were added with the Hind III restriction enzyme cutting site and the Kozak sequence GCCGCCACC at the 5' terminal, and were added with the stop codon TAG and *EcoR* I restriction enzyme cutting site at the 3' terminal, and the optimized DNA was synthesized by GenScript Biotech Corp. and cloned into a pUC57simple vector (provided by GenScript Biotech Corp.), to give pUC57simple-CD47-linker peptide-hIgG1Fc and/or CD47ECD-linker peptide-7his plasmids for human and monkey.

Followed by enzyme digestion (*Hind* III and *EcoR* I) of human and monkey plasmids pUC57simple-CD47-linker peptide-hIgG1Fc, pUC57simple-CD47ECD-linker peptide-7his and vector pcDNA3.1, the fusion gene fragments CD47-linker peptide-hIgG1Fc and CD47ECD-linker peptide-7his were recovered after electrophoresis and subjected to ligation with the pcDNA3.1 vector to construct the following expression plasmids:

- pcDNA3.1-human CD47-linker peptide-hIgG1Fc;
- pcDNA3.1-human CD47ECD-linker peptide-7his;
- pcDNA3.1-monkey CD47-linker peptide-hIgG1Fc; and
- pcDNA3.1-monkey CD47ECD-linker peptide-7his.

Gene synthesis of positive antibody (pAb) and construction of expression vector

The sequences of pAb antibodies were as follows:

PABH, as represented by SEQ ID NOs: 37 and 38; and

PABL, as represented by SEQ ID NOs: 39 and 40.

The amino acid sequences corresponding to the above antibody sequences was

codon-optimized, and added with the *Hind* III restriction enzyme cutting site and the Kozak sequence GCCGCCACC at the 5' terminal, and added with the stop codon TAG and *EcoR* I restriction enzyme cutting site at the 3' terminal, and the optimized DNA was synthesized by GenScript Biotech Corp. and cloned into a pUC57simple vector (provided by GenScript Biotech Corp.), to give pUC57simple-PCABH and pUC57simple-PCABL plasmids. Followed by enzyme digestion (*Hind* III and *EcoR* I) of plasmids pUC57simple-PCABH and pUC57simple-PCABL, the gene fragments PCABH and PCABL were recovered after electrophoresis, and then subjected to ligation with pcDNA3.1 vector to construct pcDNA3.1-PCABH and pcDNA3.1-PCABL.

1.2 Transient transfection and expression

Transient transfection and expression were performed for pcDNA3.1-PCABH, pcDNA3.1-PCABL, pcDNA3.1-human CD47-linker peptide-hIgG1Fc, pcDNA3.1-human CD47ECD-linker peptide-7his, pcDNA3.1-monkey CD47-linker peptide-hIgG1Fc, and pcDNA3.1-monkey CD47ECD-linker peptide-7his.

Transient transfection and expression were performed in a Freestyle medium using FreeStyle™ 293F cells. Twenty-four hours prior to transfection, 293F cells with a concentration of 0.6×10^6 cells/ml were inoculated in a 125 ml beaker flask and subjected to shake cultivation at 37°C and 130 rpm in a 5% CO₂ incubator. When the transfection was performed, 60 µL of 293fectin was added to 1 ml of OPTiMEM, mixed well, and incubated at room temperature for 5 minutes. At the same time, the light chains of the recombinant vectors, heavy chains of the recombinant vectors and transfection reagents were mixed at a ratio of 3:2:1 with a total DNA amount of 30 µg, and dissolved in 1 ml of OPTiMEM. Then, DNA and 293fectin were thoroughly mixed with a total volume of 2 ml, incubated at room temperature for 15 minutes, then all of the mixture was added to the cell culture wells, mixed well, and subjected to shake cultivation (at 130 rpm) at 37°C in a 5% CO₂ incubator for 7 days. The culture solution was subjected to high-speed centrifugation and vacuum filtration through a microfiltration membrane.

1.3 Protein purification

Purification was carried out using a Protein A column (protein purification liquid chromatography system/AKTA Purifier 10, GE) according to the method provided by the manufacturer to obtain a purified human PDL-1-mIgG2aFc fusion protein. An AKTA was washed with ultrapure water, and a 1 ml rProteinA Fast Flow prepacked column was connected to the AKTA. Washing: washing was performed with 5 column volumes of 1 M HAC. Equilibration: equilibration was performed with 5 column volumes of 20 mM PB 0.15 M NaCl (pH 7.0).

Loading: A cell expression supernatant sample was centrifuged at 1000 rpm for 5 min, the supernatant was taken and centrifuged at 8000 rpm for 30 min, after centrifugation, 20 ml of the supernatant was loaded, at a flow rate of 0.2 ml/min. Equilibration: equilibration was performed with 5 column volumes of 20 mM PB 0.15 M NaCl (pH 7.0) at 0.2 ml/min. Washing: washing was performed with 5 column volumes of 20 mM PB 1 M NaCl (pH 7.0). Equilibration: equilibration was performed with 5 column volumes of 20 mM PB 0.15 M NaCl (pH 7.0).

Eluting: Eluting with 20 mM sodium citrate buffer (pH 3.0) at a flow rate of 0.2 ml/min. Collection was started when 100 mAu was reached under UV 280 and stopped when absorbance value decreased to 100 mAu. The pH of the sample was adjusted to pH 6 to 8 with 1 M Tris. The purified samples were shown in Figures 1 to 2.

Example 2: Preparation of monoclonal hybridoma

1. Immunization of mice:

The immunogen human CD47-hFc (prepared in Example 1) was emulsified with adjuvant at a volume ratio of antigen to adjuvant of 1:1. A first immunization was performed by using Freund's complete adjuvant to emulsify the antigen, and after 2 weeks, a second immunization was started, the antigen was emulsified using Freund's incomplete adjuvant; immunization was performed by two-point injection, and the amount of antigen injected per mouse was 10 µg, and the volume of injection per injection point was 25 µL.

Three days after the second immunization, the mice were subjected to orbital blood collection, and a small amount of blood samples were taken for serum titer detection. After the serum titer detected by an indirect ELISA method reached 1:200,000 or above, a booster immunization is performed for the mice.

Immunizations were performed on a total of 3 groups, with 5 mice in each group.

2. Preparation of feeder cells and myeloma cells

Preparation of feeder cells: the abdominal skin of a normal BALB/C mouse (killed by cervical dislocation) was scissored to expose the peritoneum, a DMEM medium was sucked using a syringe, and injected into the peritoneal cavity of the mouse, peritoneal macrophages was washed and sucked out, and collected in a centrifuge tube for centrifugation at 1500 rmp/min for 3 min, and the lower brown precipitate was resuspended for subsequent use. The above procedures were repeated to obtain peritoneal macrophages from 3 normal mice.

Preparation of myeloma cells: P3X63Ag8.653 was thawed one week in advance, cultured in a complete medium containing 1X 8-azaguanine, and cultured with DMEM comprising 15% fetal bovine serum two days before fusion, and the confluence of P3X63Ag8.653 was maintained at 70%-80% till the day of fusion.

3. Cell fusion and HAT screening:

Acquisition and preparation of splenocytes: two mice (labeled as L1 and L2) after booster immunization was taken, after collecting the immune serums of L1 and L2, L1 and L2 were killed and immersed in 75% alcohol, the skin and peritoneum at the abdominal side of the immunized mice were scissored to expose the spleen, the surrounding tissues were removed with a scissor tip to obtain the spleen. After grinding with a grinding rod and filtering through a cell sieve, the spleen was prepared into a single cell suspension.

Pretreatment of cell fusion: P3X63Ag8.653 in a culture flask was collected, and centrifuged at 1000 rpm for 5 min, the supernatant was discarded; after resuspending, survival cells were counted, spleen cell suspension was centrifuged at 2000 rpm/5 min, the supernatant was discarded; after resuspending, survival cells was counted. The number of survival P3X63Ag8.653 cells and the number of survival spleen cells were recorded.

Cell fusion: the cells was mixed according to a ratio of spleen cells to P3X63Ag8.653 of 5:1, centrifuged at 2000 rpm for 5 min, the supernatant was discarded, the cell precipitate was shaken to disperse; 1 mL of preheated 50% PEG1500 solution was slowly dropwise added in a 37°C water bath while swaying the bottom of the tube in the water at 37°C, with the above operation time being controlled at about 1 min; After standing for reacting for 30 s, a DMEM medium preheated at 37°C was added into the tube in a manner from slow to fast addition,

then the reaction was terminated. The cell suspension was centrifuged at 800 rpm for 3 min after the termination of the reaction, the supernatant was discarded, and the cell precipitate was gently shaken to disperse.

HAT medium screening: a HAT screening medium containing 1×HAT, 1×penicillin-streptomycin, 15% fetal bovine serum and 85% DMEM medium was prepared, mouse hybridoma cells and feeder cells were respectively resuspended in the above HAT screening medium, and then mixed. The obtained cell suspension was added into twenty 96-well cell culture plates at 300 µL/well, and cultured in a cell culture incubator at 37°C. After one week of culturing, the cell culture medium was replaced with HT medium for the first time, then cultured in a cell culture incubator at 37°C, and after three days of culturing, the culture medium was replaced HT medium for the second time.

4. Screening for positive cell strains

Two weeks after the fusion, the cell supernatant was taken for a ELISA assay, and the binding status of the cell supernatant with the human CD47-his protein was detected. After the cells with positive ELISA results were selected, a second ELISA assay was performed. The cell supernatant with positive retest results was subjected to a FACS assay to detect the binding status of the cell supernatant to the CD47 protein on the surface of jurkat cells.

5. Amplification culture

The cell strain detected as positive in both ELISA and FACS assays was transferred from a 96-well plate to a 24-well plate for culturing, and after confluence, transferred to a culture flask of 25 cm² for culturing.

6. Subcloning by limited dilution

The positive cell strains were mixed well by repeated pipetting, and a small amount was taken for survival cells count. About 100 cells were added into 40 mL of complete medium, mixed well, and sown in two plates. Additionally, about 100 cells were added into 20mL of complete medium, mixed well and sown in one plate. About 1000 cells were added into 20mL of complete medium, mixed well and sown in one plate. Samples were sown in totally four plates at three different cell densities: 0.5 cell/well, 1 cell/well, and 10 cells/well. The 96-well plates were placed in a 5% CO₂ incubator at 37°C for culturing.

7. Clone detection and amplification culture

The supernatant of the monoclonal cell well was taken for ELISA and FACS assay, and the binding status of the cell clonal antibody with the human and monkey CD47-his label and the binding status with the CD47 protein on the surface of jurkat cells were detected, respectively. The positive cell strains detected as positive in both ELISA and FACS (7 cell strains in total, respectively labeled as 059-1.11.1, 059-1.20.1, 059-1.30.1, 059-1.43.1, 059-1.51.2, 059-1.82.1, 059-1.100.5) were transferred from the 96-well plate to a 24-well plate for culturing, and after confluence, transferred to a 25 cm² culture flask for culturing.

8. Identification of subclasses

A plate was coated with goat-anti-mouse IgG (Fc) at 2 µg/ml, 50 µL per well, stayed overnight at 4°C, and blocked with BSA; the supernatant of the cells to be detected was added at room temperature and incubated for 2 hours, enzyme-labeled subclass secondary antibodies IgG1, IgG2a, IgG2b, IgG2c, IgG3, κ, and λ (abcam) were added, color development was performed, and the absorbance was read at 450 nm, the subclass of the detected cell strains was determined as IgG3 or IgM or IgG1, and κ.

Wherein, the heavy chain constant region of the antibody 059-1.82.1 was mouse IgG3, the light chain constant region was the constant region of the mouse κ chain; the heavy chain constant regions of 059-1.30.1, 059-1.43.1 and 059-1.20.1 were mouse IgG1, the light chain constant regions were the constant region of the mouse κ chain; the heavy chain constant regions of 059-1.11.1, 059-1.51.2, and 059-1.100.5 were mouse IgM. The amino acid sequence of the heavy chain variable region was one of SEQ ID NOs: 1 to 7, and the amino acid sequence of the light chain variable region was one of SEQ ID NOs: 8 to 14.

Washing was performed with PBST for three times before each adding solution step.

9. Cell cryopreservation

Preparation of cryopreservation solution: 90% fetal bovine serum, 10% DMSO.

The cells in a culture flask were resuspended. After the cells were counted, centrifugation was performed at 1500 rpm/min for 3 min, and the supernatant was discarded; followed by suspending with fetal bovine serum containing 10% DMSO by repeated pipetting, the obtained suspension was cryopreserved in a cryopreservation box with 5×10⁶ cells per tube, stayed overnight at -80°C, and transferred into liquid nitrogen on the next day.

10. Preservation of genes of monoclonal hybridoma

RNA was extracted from a positive monoclonal cell strain RNA extraction solution, and was reverse transcribed into cDNA, which was permanently stored at -80°C.

Example 3 Preparation and identification of monoclonal antibodies

1. Preparation of antibodies by in vitro culture

The hybridoma cell strain prepared in Example 2 was thawed, and the method for thawing was as follows: the DMEM medium containing 10% fetal bovine serum and 1% penicillin-streptomycin was thawed and cultured in a vial, after the cell confluence reaches about 90%, passage amplification culture was performed until a total of about 200 mL of the cell culture supernatant was reached.

2. Antibody purification

When cells were cultured for about 7 days, the cell supernatant was collected, the volume (about 200 mL) thereof was measured, NaCl was added to the supernatant to a concentration of 2.5 M; after vacuum filtering through a 0.22 µm mixed cellulose microfiltration membrane, the supernatant was stored at 4°C, and antibody purification was performed by Protein A affinity chromatography. Loading: the cell culture supernatant containing 2.5 M NaCl was filtered through a 0.22 µm filtration membrane, concentrated to 30 ml, and then directly loaded; washing: washing with 2.5 M PBS (pH 7.4) till UV280 baseline being 0; eluting: eluting with 0.1 M citric acid solution (pH 3.5). The eluent was collected in 2 ml for each fraction, 100 µL of 1 M Tris solution was added to each tube; and the collected solution was concentrated. Elution was performed with PBS until the ratio of the initial component was less than 0.1%. SDS-PAGE method was carried out to verify the purity of the purified antibody (Figure 3).

Example 4 Gene sequencing of monoclonal antibody and preparation of recombinant Antibody

1. Gene sequencing of monoclonal antibody

After immunization, fusion and monoclonalization, 059-1.11.1, 059-1.20.1, 059-1.30.1, 059-1.43.1, 059-1.51.2, 059-1.82.1, 059-1.100.5 were selected based on the results of affinity experiments, total RNA of monoclonal antibody cell strains was extracted, and reverse transcribed into cDNA, and then the heavy chain variable region and the light chain variable region of the antibody were amplified using PCR with the cDNA as a template.

Sequence analysis of the heavy chain and the light chain of the antibody genes. The total RNA of 7 monoclonal antibody cell strain was extracted using Invitrogen's Trizol® reagent kit (15596-026) according to the instructions thereof. The extraction results were shown in Figure 4.

5 Then, using Takara's 5'RACE FULL kit (D315), with the total RNA as a template, reverse transcription was performed to give a first chain cDNA using the random primers in the kit, then amplification of the heavy chain was performed using PCR with a primer designed for the constant region (mIgG R) and a linker primer in the kit, and the amplification of the light chain was performed using PCR with a primer designed for constant region (mIgK
10 R) and a linker primer in the kit. The sequences of mIgG R and mIgK R were as follows:

mIgG R: CTCAGGGAARTARCCYTTGAC (as represented by SEQ ID NO: 41); and

mIgK R: TCACTGCCATCAATCTTCCAC (as represented by SEQ ID NO: 42).

The electrophoretic detection of the heavy chain variable region and the light chain variable region of the 7 monoclonal antibody cell strains after PCR amplification were shown
15 in Figure 5.

The PCR fragment was recovered using an agarose gel recovery kit, then TA cloning was performed, and a single clone was selected for PCR identification. The primers for identification were M13-F and M13-R, and some samples selected from the cell strains identified as correct were sent to Invitrogen for sequencing. The protein sequences of the
20 heavy chain variable region were finally determined to be SEQ ID NOs: 1 to 7; the protein sequences of the light chain variable region were SEQ ID NOs: 8 to 14; the heavy chain nucleotide sequences were SEQ ID NOs: 15 to 21; and the light chain nucleotide sequences were SEQ ID NOs: 22 to 28.

Identification primer M13-F was as follows:

25 5'TGTAAAACGACGGCCAGT3' (as represented by SEQ ID NO: 43).

Identification primer M13-R was as follows:

5'CAGGAAACAGCTATGACC3', (as represented by SEQ ID NO: 44).

Table 1: Sequences

	Amino acid sequences		Nucleotide sequences	
	heavy chain variable region	light chain variable region	heavy chain variable region	light chain variable region
059-1.100.5	SEQ ID NO: 1	SEQ ID NO: 8	SEQ ID NO: 15	SEQ ID NO: 22
059-1.82.1	SEQ ID NO: 2	SEQ ID NO: 9	SEQ ID NO: 16	SEQ ID NO: 23
059-1.51.2	SEQ ID NO: 3	SEQ ID NO: 10	SEQ ID NO: 17	SEQ ID NO: 24
059-1.43.1	SEQ ID NO: 4	SEQ ID NO: 11	SEQ ID NO: 18	SEQ ID NO: 25
059-1.30.1	SEQ ID NO: 5	SEQ ID NO: 12	SEQ ID NO: 19	SEQ ID NO: 26
059-1.20.1	SEQ ID NO: 6	SEQ ID NO: 13	SEQ ID NO: 20	SEQ ID NO: 27
059-1.11.1	SEQ ID NO: 7	SEQ ID NO: 14	SEQ ID NO: 21	SEQ ID NO: 28

Example 5 Affinity determination and competitive ELISA assay

1. Detection of binding with human CD47 (ELISA)

5 Coating: hCD47-his was diluted to 1 µg/ml with PBS, added to 96 wells of an ELISA plate with 100 µL per well, and incubated overnight at 4°C. Blocking: after the plate was washed for three times, it was blocked with 1% BSA + PBS at 300 µL per well, and incubated at room temperature for 2 hours. Addition of candidate antibody: after the plate was washed for three times, the cell culture supernatant of the candidate antibody or a positive control or a

10 negative control was added at 100 µL per well, and incubated at room temperature for 2 hours. Addition of secondary antibody: after the plate was washed for three times, goat-anti-mouse IgG Fc and HRP (1:10000) were added at 100 µL per well, and reacted at room temperature for 1 hour. Color development: after the plate was washed for four times, a TMB color developing solution was added at 100 µL per well, followed by color development at room

15 temperature in the dark for 10 minutes. Termination: the reaction was terminated by directly adding a stop solution with 100 µL per well. Detection: immediately after termination of the reaction, the ELISA plate was placed in an ELISA reader, and the OD value was measured at 450 nm. The original data was saved as shown in Table 2 below:

Table 2: ELISA detected OD450 values of candidate clones and human CD47

Antibody name	OD450
059-1.11.1	3.6718
059-1.20.1	4.0

059-1.30.1	4.0
059-1.43.1	4.0
059-1.51.2	0.7709
059-1.82.1	4.0
059-1.100.5	0.81
Negative control	0.086

2. Detection of binding with monkey CD47 (ELISA)

Coating: Monkey CD47-his was diluted to 1 µg/ml with PBS, added to 96 wells of an ELISA plate at 100 µL per well, and incubated overnight at 4°C. Blocking: after the plate was washed for three times, it was blocked with 1% BSA + PBS at 300 µL per well, and incubated at room temperature for 2 hours. Addition of candidate antibody: after the plate was washed for three times, the cell culture supernatant of the candidate antibody or a negative control was added at 100 µL per well, and incubated at room temperature for 2 hours. Addition of secondary antibody: after the plate was washed for three times, goat-anti-mouse IgG Fc and HRP (1:10000) were added at 100 µL per well, and reacted at room temperature for 1 hour. Color development: after the plate was washed for four times, a TMB color developing solution was added at 100 µL per well, followed by color development at room temperature in the dark for 10 minutes. Termination: the reaction was terminated by directly adding a stop solution at 100 µL per well. Detection: immediately after termination of the reaction, the ELISA plate was placed in an ELISA reader, and the OD value was measured at 450 nm. The original data was saved as shown in Table 3 below:

Table 3: ELISA detected OD values of candidate clones and monkey CD47

Antibody name	OD450
059-1.11.1	4
059-1.20.1	4
059-1.30.1	4
059-1.43.1	4
059-1.51.2	0.6336
059-1.82.1	3.896
059-1.100.5	0.6166
Negative control	0.0883

3. Detection of affinity with human CD47 protein

The affinity constant of the human CD47 antibody was detected using a Biacore T200 instrument; an anti-mouse Fc antibody (GE Healthcare Company, BR-1008-38) was coupled to a CM5 biosensor chip (GE Healthcare Company) via amino covalent binding, the anti-mouse Fc antibodies on the chip captured candidate monoclonal antibodies or positive control B6H12 (commercialized CD47 antibody, purchased from Abeam, catalog No. ab3283); human CD47 with different concentrations was flowed through the candidate antibody on the chip at a flow rate of 30 $\mu\text{L}/\text{min}$, and human CD47 bound with the candidate antibody with a binding time of 120 s and a dissociation time of 300 s. A dynamic fitting was performed using BIAevaluation software (GE Healthcare Company), and 059-1.82.1 had the highest affinity. The obtained affinity constants were shown in Table 4 below.

Table 4: Determination results of affinity between candidate clones and human CD47

Antibody name	Ka (1/Ms)	Kd(1/s)	KD(nM)
059-1.11.1	2.60E5	0.004015	15.5
059-1.20.1	2.17E5	0.00548	25.3
059-1.30.1	4.24E5	0.01276	30.1
059-1.43.1	2.36E5	0.006657	28.3
059-1.51.2	3.70E5	0.01009	27.3
059-1.82.1	2.56E5	0.003643	14.2
059-1.100.5	1.57E5	0.0069	44.0
Positive control	1.32E5	0.003636	27.5

Example 6 Dose-dependent blocking of the binding between human CD47 and human SIRP (ELISA method)

Coating: Human CD47-hFc was diluted to 2 $\mu\text{g}/\text{ml}$ with PBS, added to 96 wells of an ELISA plate at 100 μL per well, and incubated overnight at 4°C. Blocking: after the plate was washed for three times, it was blocked with 1% BSA + PBS at 300 μL per well, and incubated at room temperature for 1 hour. Mixing of candidate antibodies with SIRP-his: purified candidate antibodies were diluted to 20 $\mu\text{g}/\text{ml}$ with PBST, and a 3-fold gradient dilution was performed with PBST solution, totally 7 gradients; human SIRP-his protein was diluted to

500 ng/mL with PBST, and the candidate antibodies with different dilution gradients were mixed with human SIRP-his protein at a ratio of 1:1, and incubated at room temperature for 30 min. Addition of a mixture of the candidate antibodies and human SIRP-his protein: 100 μ L per well, reaction is performed at room temperature for 1 hour, and a mixture of a IgG isotype control and human SIRP-his protein was added to a control well. Addition of secondary antibody: after the plate was washed for three times, an anti-His tag antibody and HRP (1:3000) were added at 100 μ L per well, and reacted at room temperature for 1 hour. Color development: after the plate was washed for four times, a TMB color developing solution was added at 100 μ L per well, followed by color development at room temperature in the dark for 10 minutes. Termination: the reaction was terminated by directly adding a stop solution at 100 μ L per well. Detection: immediately after termination of the reaction, the ELISA plate was placed in an ELISA reader, the OD value was measured at 450 nm, and the original data was saved. Data Processing: the original data was inputted into software SoftMax Pro 6.2.1 for data processing. The results were shown in Table 5 and Figure 6 (the data in the figure was the final calculated data).

Table 5: Results about blocking the binding between CD47 and SIRP for candidate antibody

Antibody name	EC50 (nM)
059-1.11.1	2340
059-1.20.1	1190
059-1.30.1	1030
059-1.43.1	850
059-1.51.2	--
059-1.82.1	1030
059-1.100.5	--
Positive control	1190
Negative control	--

The results showed that the candidate antibodies 059-1.11.1, 059-1.20.1, 059-1.30.1, 059-1.43.1 and 059-1.82.1 all had strong blocking effects, and 059-1.51.2 and 059-1.100.5 had no effects on blocking the binding of human CD47 to human SIRP.

Example 7 Determination of binding to CD47 on cell surface (FACS method)

Jurkat cells were cultured and collected, subjected to centrifugation at 2000 rpm for 5 min, and washed once with PBS, and then survival cells was counted ; 2.0×10^5 cells were added to each tube, and centrifuged at 2000 rpm for 5 min, the supernatant was discarded, 100 μ L supernatant of candidate antibody cells was added to each tube, 100 μ L of 1 ng/mL B6H12 antibody (commercialized CD47 antibody, purchased from Abcam, catalog No.: ab3283) was added to a positive control tube, 100 μ L of DMEM medium was added to a negative control tube, and reacted at room temperature for 1 hour; centrifugation was carried out at 2000 rpm for 5 min, washing with PBS was performed for 3 times, 100 μ L of 4 ng/mL goat-anti-mouse IgG Fc and FITC secondary antibody were added to each tube, and reacted at room temperature for 1 hour; centrifugation was carried out at 2000 rpm for 5 min, washing with PBS was performed for 3 times, 300 μ L of PBS was added to each tube, and the obtained sample was transferred to a sample injection tube of a flow cytometer, and detection was performed on the flow cytometer. The results were shown in Table 6 and Figure 7.

Table 6: Results about binding of candidate antibodies with CD47 on the surface of Jurkat cells

Antibody name	MFI
059-1.11.1	6633
059-1.20.1	15315
059-1.30.1	9951
059-1.43.1	18533
059-1.51.2	144
059-1.82.1	9547
059-1.100.5	216
Positive control (B6H12)	13000
Negative control	57

The results showed that the candidate antibodies had significant binding activity to the CD47 on cell surface.

Example 8 Experiments about CD47 antibody mediated phagocytosis of Jurkat Cells by mouse peritoneal primary macrophages

1. Preparation of C57 mouse peritoneal primary macrophages

The animal was killed by cervical dislocation, with the mouse tail in hand, the whole mouse was immersed in 70% alcohol for 3 to 5 seconds. The animal was placed on a dissection table, the limbs were fixed with needles, with both hands holding forceps, the skin was torn apart and pulled to two sides to expose the peritoneum, the peritoneal wall was scrubbed with 70% alcohol, and then 10ml of Eagle solution was injected into the abdominal cavity with a syringe, while the peritoneal wall was kneaded with fingers from both sides to allow the liquid to flow sufficiently in the abdominal cavity. The abdominal wall was gently picked up with a needle, so that the animal body slightly leaned to one side, and the liquid in the abdominal cavity was collected and sucked into the needle tube. The needle was carefully pulled out, and the liquid was injected into a centrifuge tube. Followed by centrifugation (250 g) at 4°C for 4 minutes, the supernatant was discarded, and 10 ml of Eagle medium was added, and survival cells were counted. In order to obtain 3×10^5 attached cells per square centimeter, cells were inoculated at a final concentration of 2.5×10^6 /ml. In order to purify the cultured cells and remove other leukocyte, several hours after inoculation, the culture solution was removed, the rest was washed with Eagle solution for 1 to 2 times, then a fresh Eagle culture solution was added, and cultivation was performed at 37°C in a 5% CO₂ incubator.

2. Macrophage phagocytosis experiment

The effect of CD47 antibody prepared in Example 5 on the phagocytosis of Jurkat cells by C57BL mouse peritoneal primary macrophages was examined by fluorescence imaging. Mouse macrophages were stained with PKH26 dye one day in advance (4 micromoles, 5 min), and inoculated in a 96-well plate at 20,000 cells/well; the next day, Jurkat cells (2 micromoles, 10 min) were stained with CFSE dye, after washing, the Jurkat cells were resuspended in a serum-free medium and added to macrophages at 80,000 cells/well. 2 hours before the Jurkat cells were inoculated, the serum-containing medium for macrophages was replaced with a serum-free medium and various antibodies were added to the suspension of two cells at a concentration of 10 µg/ml. After 2 hours of culturing, Jurkat cells that were not phagocytized were washed away, and after cell imaging by fluorescence microscopy, the level of the effect of CD47 antibody on promoting phagocytosis were quantified by counting how many Jurkat cells were phagocytized per 100 macrophages, i.e., phagocytic index. The results were shown in Figure 8 (a graph showing phagocytic effect) and Table 7 (phagocytic index). As reported

by literatures, B6H12 (commercialized CD47 antibody, purchased from Abcam, catalog No.: ab3283) has a weaker effect on promoting phagocytosis. Four selected antibody molecules in Example 5 have a good effect on promoting phagocytosis, especially 059-1.30.1, 059-1.43.1, and 059-1.82.1.

Table 7: CD47 antibodies promote macrophage phagocytosis of Jurkat cells

Antibody name	phagocytic index
059-1.20.1	43
059-1.30.1	70
059-1.43.1	79
059-1.82.1	74
Positive control (B6H12)	20
Negative control	0

Example 9 In vitro induction of apoptosis of Jurkat cells by CD47 antibody

Jurkat cells at logarithmic growth phase were collected, and washed with a serum-free medium, a single cell suspension was prepared with 5% FBS-1640, and the cells were resuspended to $10 \times 10^5/\text{ml}$, and added to a 24-well plate for culturing at $5 \times 10^5/\text{well}$, i.e., 500 $\mu\text{L}/\text{well}$. The experiment was designed as follows: CD47 antibody (final concentration: 10 $\mu\text{g}/\text{ml}$) was added at 50 $\mu\text{L}/\text{well}$, an anti-Fas positive control well was set, and the same volume of medium was added to the wells without the addition of antibodies. After 5 h, the cell suspension was collected into a 1.5 ml EP tube and centrifuged ($500 \text{ g} \times 5 \text{ min}$). The supernatant was discarded, cells were resuspended with 100 μL of PBS and mixed well in each tube, and the cells were stained with annexin-V (Roche Diagnostics) at 4°C in the dark for 30 minutes. Washing with PBS was performed for three times, 500 μL of PBS was added to each tube to resuspend and mix cells, and PI (final concentration of 1 $\mu\text{g}/\text{ml}$) was added 10 to 15 min before loading on a flow cytometer. Measuring the ratio of annexin-V positive cells and annexin-V and PI double positive cells to the total cells (i.e., the apoptotic rate of Jurkat cells) was performed on the flow cytometer.

Table 8: CD47 antibody induced apoptosis of Jurkat cells

Antibody name	Apoptotic rate (%)
059-1.20.1	33
059-1.30.1	48

059-1.43.1	37
059-1.82.1	24
Control antibody (B6H12)	8
Negative control	5

5 B6H12 did not induce apoptosis of Jurkat cells, while four tested 059 antibodies showed induction of apoptosis of Jurkat cells in different degrees, and 059-1.30.1 had the strongest effect. 059-1.30.1 showed the best effect on promoting macrophage phagocytosis and inducing apoptosis of Jurkat cells.

The above are only preferred embodiments of the present invention, and it should be noted that a person skilled in the art can also make various improvements and modifications without departing from the principles of the present invention. These improvements and
10 modifications should also be considered to be within the scope of protection of the present invention.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive
15 sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

SEQUENCE LISTING

<110> CHANGCHUN GENESCIENCE PHARMACEUTICALS CORP.

<120> Anti-CD47 monoclonal antibody and use thereof

<130> OP170553

5 <160> 62

<170> PatentIn version 3.3

<210> 1

<211> 136

10 <212> PRT

<213> Artificial sequence

<400> 1

Met Glu Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Val Ile Ala Gly

1 5 10 15

15 Val Gln Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg

20 25 30

Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe

35 40 45

Thr Asp Tyr Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu

20 50 55 60

Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn

65 70 75 80

Gln Asn Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser

85 90 95

25 Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val

100 105 110

Tyr Tyr Cys Thr Arg Gly Thr Pro Tyr Ala Met Asp Tyr Trp Gly Gln

115 120 125

Gly Thr Ser Val Thr Val Ser Ser

30 130 135

<210> 2

<211> 136

<212> PRT

35 <213> Artificial sequence

<400> 2

Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly

1 5 10 15
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30
 Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 5 35 40 45
 Thr Asn Tyr Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60
 Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Asn Tyr Asn
 65 70 75 80
 10 Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser
 85 90 95
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110
 Tyr Tyr Cys Ala Lys Gly Gly Trp Phe Ser Met Asp Tyr Trp Gly Gln
 15 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser
 130 135

 <210> 3
 20 <211> 136
 <212> PRT
 <213> Artificial sequence
 <400> 3
 Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 25 1 5 10 15
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30
 Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 30 Thr Asn Tyr Val Met His Trp Met Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60
 Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Asp Thr Lys Tyr Asn
 65 70 75 80
 Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser
 35 85 90 95
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110

Tyr Tyr Cys Ala Lys Gly Gly Tyr Tyr Ser Met Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser
 130 135

5

<210> 4
 <211> 136
 <212> PRT
 <213> Artificial sequence

10

Met Glu Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Val Ile Ala Gly
 1 5 10 15
 Val Gln Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
 20 25 30

15

Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr Asp Tyr Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu
 50 55 60

20

Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn
 65 70 75 80
 Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
 85 90 95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110

25

Tyr Tyr Cys Thr Arg Gly Thr Pro Tyr Ala Met Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser
 130 135

30

<210> 5
 <211> 136
 <212> PRT
 <213> Artificial sequence
 <400> 5

35

Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys

20 25 30
 Pro Gly Ala Ser Val Arg Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr Asn Tyr Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 5 50 55 60
 Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Ser
 65 70 75 80
 Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile
 85 90 95
 10 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110
 Tyr Tyr Cys Ala Lys Gly Gly Leu Phe Thr Phe Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Thr Leu Thr Val Ser Ser
 15 130 135

 <210> 6
 <211> 136
 <212> PRT
 20 <213> Artificial sequence
 <400> 6
 Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 25 20 25 30
 Pro Gly Ala Ser Val Asn Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr Asn Tyr Phe Leu His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60
 30 Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Asn Tyr Asn
 65 70 75 80
 Glu Asn Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser
 85 90 95
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 35 100 105 110
 Tyr Tyr Cys Ala Lys Gly Gly Trp Phe Ser Met Asp Tyr Trp Gly Gln
 115 120 125

Gly Thr Ser Val Thr Val Ser Ser
130 135

5 <210> 7
<211> 136
<212> PRT
<213> Artificial sequence
<400> 7

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

20 Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
85 90 95
Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
100 105 110
Tyr Tyr Cys Thr Arg Gly Thr Pro Tyr Ala Met Asp Tyr Trp Gly Gln
25 115 120 125
Gly Thr Ser Val Thr Val Ser Ser
130 135

30 <210> 8
<211> 131
<212> PRT
<213> Artificial sequence
<400> 8

35 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1 5 10 15
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 35 40 45
 Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
 50 55 60
 5
 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
 65 70 75 80
 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 85 90 95
 10 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 100 105 110
 Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
 115 120 125
 Glu Ile Lys
 15 130
 <210> 9
 <211> 130
 <212> PRT
 20 <213> Artificial sequence
 <400> 9
 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 1 5 10 15
 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 25 20 25 30
 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 35 40 45
 Val His Ser Asn Gly Lys Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro
 50 55 60
 30 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
 65 70 75 80
 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 85 90 95
 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 35 100 105 110
 Ser Gln Ser Thr His Val Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
 115 120 125

Ile Lys

130

<210> 10

5 <211> 131

<212> PRT

<213> Artificial sequence

<400> 10

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala

10 1 5 10 15

Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val

20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu

35 40 45

15 Val His Ser Lys Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro

50 55 60

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

65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr

20 85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys

100 105 110

Ser Gln Ser Thr His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu

115 120 125

25 Glu Ile Lys

130

<210> 11

<211> 131

30 <212> PRT

<213> Artificial sequence

<400> 11

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala

1 5 10 15

35 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val

20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu

35 40 45
 Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
 50 55 60
 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
 5 65 70 75 80
 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 85 90 95
 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 100 105 110
 10 Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
 115 120 125
 Glu Ile Lys
 130
 15 <210> 12
 <211> 131
 <212> PRT
 <213> Artificial sequence
 <400> 12
 20 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 1 5 10 15
 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 20 25 30
 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 25 35 40 45
 Val His Ser Lys Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
 50 55 60
 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser
 65 70 75 80
 30 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 85 90 95
 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 100 105 110
 Ser Gln Ser Thr His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
 35 115 120 125
 Glu Ile Lys
 130

<210> 13
 <211> 130
 <212> PRT
 5 <213> Artificial sequence
 <400> 13
 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 1 5 10 15
 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 10 20 25 30
 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 35 40 45
 Val His Ser Asn Gly Lys Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro
 50 55 60
 15 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
 65 70 75 80
 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 85 90 95
 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
 20 100 105 110
 Ser Gln Ser Thr His Val Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
 115 120 125
 Ile Lys
 130
 25
 <210> 14
 <211> 131
 <212> PRT
 <213> Artificial sequence
 30 <400> 14
 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 1 5 10 15
 Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 20 25 30
 35 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
 35 40 45
 Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro

	50	55	60	
	Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser			
	65	70	75	80
	Gly Val Pro Glu Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr			
5		85	90	95
	Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys			
	100	105	110	
	Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu			
	115	120	125	
10	Glu Ile Lys			
	130			
	<210> 15			
	<211> 408			
15	<212> DNA			
	<213> Artificial sequence			
	<400> 15			
	atggaatgga gctgggtcctt tctcttctc ctgtcagtaa ttgcaggtgt ccaatcccag	60		
	gttcaactgc agcagtctgg ggctgagctg gtgaggcctg gggcttcagt gacgtgtcc	120		
20	tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct	180		
	gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggtggtac tgcctacaat	240		
	cagaacttca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg	300		
	gagctccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc	360		
	tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctctca	408		
25				
	<210> 16			
	<211> 408			
	<212> DNA			
	<213> Artificial sequence			
30	<400> 16			
	atggaatgga gttggatatt tctcttctc ctgtcaggaa ctgcaggtgt ccactctgag	60		
	gtccagctgc agcagtctgg acctgagctg gtaaagcctg gggcttcagt gaagatgtcc	120		
	tgcaaggctt ctggatacac attactaac tatgttatgc actgggtgaa gcagaagcct	180		
	ggacagggcc ttgagtggat tggatatatt aatccttaca atgatggtac taactacaat	240		
35	gagaagtta aaggcaaggc cacactgact tcagacaaat cctccagcac agcctacatg	300		
	gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaa aggggggtgg	360		
	ttctccatgg actactgggg tcaaggaacc tcagtcaccg tctctca	408		

	<210>	17	
	<211>	408	
	<212>	DNA	
5	<213>	Artificial sequence	
	<400>	17	
	atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag	60	
	gtccagctgc agcagctctgg acctgagctg gtaaagcctg gggcttcagt gaagatgtcc	120	
	tgcaaggctt ctggatacac attcactaac tatgttatgc actggatgaa gcagaagcct	180	
10	gggcagggcc ttgagtggat tggatatatt aatccttaca atgatgatac taaatacaat	240	
	gagaaattca aagacagggc cacactgact tcagacaaat cctccagcac agcctacatg	300	
	gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa ggggggttac	360	
	tattctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca	408	
15	<210>	18	
	<211>	408	
	<212>	DNA	
	<213>	Artificial sequence	
	<400>	18	
20	atggaatgga gctgggtctt tctcttctc ctgtcagtaa ttgcaggtgt ccaatcccag	60	
	gttcaactgc agcagctctgg ggctgagctg gtgaggcctg gggcttcagt gacgtgtcc	120	
	tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct	180	
	gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggtgttac tgcctacaat	240	
	cagaagtca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg	300	
25	gaactccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc	360	
	tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca	408	
30	<210>	19	
	<211>	408	
	<212>	DNA	
	<213>	Artificial sequence	
	<400>	19	
	atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag	60	
	gtccagctgc agcagctctgg acctgagctg gtaaagcctg gggcttcagt gaggatgtcc	120	
35	tgcaaggctt ctggatacac attcactaac tatgttatgc actgggtgaa gcagaagcct	180	
	gggcagggac ttgagtggat tggatatatt aatccttaca atgatgttac taagtacagt	240	
	gagaagtca aaggcaaggc cacactgact tcagacaaat cctccatcac agcctatatg	300	

	gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa aggggggctt	360
	tttacctttg actactgggg ccaaggcacc actctcacag tctctca	408
	<210> 20	
5	<211> 408	
	<212> DNA	
	<213> Artificial sequence	
	<400> 20	
	atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag	60
10	gtccagctgc agcagctcgg acctgagctg gtaaagcctg gggcttcagt taatatgtcc	120
	tgcaaggctt ctggatacac attactaac tattttttgc actgggtgaa gcagaagcct	180
	gggcagggcc ttgagtggat tggatatatt aatccttaca atgatgggtac taactacaat	240
	gagaacttca aaggcaaggc cactctgact tcagacaaat cctccagcac agcctacatg	300
	gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa aggggggtgg	360
15	tttccatgg actactgggg tcaaggaacc tcagtcaccg tctctca	408
	<210> 21	
	<211> 408	
	<212> DNA	
20	<213> Artificial sequence	
	<400> 21	
	atggaatgga gctgggtctt tctcttctc ctgtcagtaa ttgcaggtgt ccaatcccag	60
	gttcaactgc agcagctcgg ggctgagctg gtgaggcctg gggcttcagt gacgtgtcc	120
	tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct	180
25	gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggtggtac tgcctacaat	240
	cagaagtca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg	300
	gagctccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc	360
	tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctctca	408
30	<210> 22	
	<211> 393	
	<212> DNA	
	<213> Artificial sequence	
	<400> 22	
35	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgtctc cagcagtgat	60
	gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc	120
	tcttcagat ctagtcatg cctgttacac agtaatggaa acacctattt acattggtac	180

	ctgcagaagc caggccagtc tccaaagctc ctgactaca aagtttcaa cgcattttct	240
	ggggtcccag acaggttcag tggcagtga tcaggacag atttcacact caagatcagc	300
	agagtggagg ctgaggatct gggagtttat ttctgctctc aaaglacaca tgtccgtgg	360
	acgttcggtg gaggcaccaa gctggaaatc aaa	393
5		
	<210> 23	
	<211> 390	
	<212> DNA	
10	<213> Artificial sequence	
	<400> 23	
	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat	60
	gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc	120
	tcttcagat ctatgcagag ccttgtacac agtaatggaa aaacctattt acagtggtag	180
15	ctgcagaagc caggccagtc tccaaagctc ctgactaca aagtttcaa cgcattttct	240
	ggggtcccag acaggttcag tggcagtga tcaggacag atttcacact caagatcagc	300
	agagtggagg ctgaggatct gggagtttat ttctgctctc aaaglacaca tgtttacacg	360
	ttcggagggg ggaccaagct ggaaataaaa	390
20		
	<210> 24	
	<211> 393	
	<212> DNA	
	<213> Artificial sequence	
	<400> 24	
25	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat	60
	gttgtgatga cccaaactcc acttctctg cctgtcagtc ttggagatca agcctccatc	120
	tcttcagat ctatgcagag ccttgtacac agtaaaggaa acacctattt acattggtag	180
	ctgcagaagc caggccagtc tccaaagctc ctgactaca aagtttcaa cgcattttct	240
	ggggtcccag acaggttcag tggcagtga tcaggacag atttcacact caagatcagc	300
30	agagtggagg ctgaggatct gggagtttat ttctgctctc aaaglacaca tgttccattc	360
	acgttcggtc cggggacaaa gttggaaata aaa	393
35		
	<210> 25	
	<211> 393	
	<212> DNA	
	<213> Artificial sequence	
	<400> 25	

	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat	60
	gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc	120
	tcttcagat ctagtcagag cctgttacac agtaatggaa acacctattt acattggtac	180
	ctgcagaagc caggccagtc tccaaagctc ctgacttaca aagtttccaa ccgattttct	240
5	ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc	300
	agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgtccgtgg	360
	acgttcgggtg gaggcaccaa gctggaaatc aaa	393
	<210> 26	
10	<211> 393	
	<212> DNA	
	<213> Artificial sequence	
	<400> 26	
	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat	60
15	gttgtgatga cccaaactcc actctccctg cctgtcagcc ttggagatca agcctccatc	120
	tcttcagat ctagtcagag cctgttacac agtaaaggaa acacctattt acattggtac	180
	ctgcagaagc caggccagtc tccaaagctc ctgacttaca gagtgtccaa ccgattttct	240
	ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc	300
	agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccattc	360
20	acgttcggct cggggacaaa gttggaaata aaa	393
	<210> 27	
	<211> 390	
	<212> DNA	
25	<213> Artificial sequence	
	<400> 27	
	atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat	60
	gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc	120
	tcttcagat ctagtcagag cctgttacac agtaatggaa aaacctattt acagtgggtac	180
30	ctgcagaagc caggccagtc tccaaagctc ctgacttaca aagtttccaa ccgattttct	240
	ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc	300
	agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgtttacacg	360
	ttcggagggg ggaccaagct ggaaataaaa	390
35	<210> 28	
	<211> 393	
	<212> DNA	

<213> Artificial sequence

<400> 28

atgaagttgc ctgttaggct gttggtgctg atgttctgga ttctgcttc cagcagtgat 60
 gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc 120
 5 tcttcagat ctagtcagag cctgttacac agtaatggaa acacctattt acattggtac 180
 ctgcagaagc caggccagtc tccaaagctc ctgactaca aagtttcaa ccgattttct 240
 ggggtcccag aaagggtcag tggcagtggg tcagggacag atttcacact caagatcagc 300
 agagtggagg ctgaggatct gggagtatat ttctgctc aaaglacaca tgtccgtgg 360
 acgttcggtg gaggcaccaa gctggaaatc aaa 393

<210> 29

<211> 379

<212> PRT

<213> Artificial sequence

<400> 29

Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly

1 5 10 15

Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe

20 25 30

20 Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala

35 40 45

Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp

50 55 60

Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Val Pro Thr Asp

25 65 70 75 80

Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala

85 90 95

Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr

100 105 110

30 Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu

115 120 125

Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly

130 135 140

Ser Ala Ser Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro

35 145 150 155 160

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro

165 170 175

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 180 185 190
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 195 200 205
 5 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 210 215 220
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 225 230 235 240
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 10 245 250 255
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 260 265 270
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 275 280 285
 15
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 290 295 300
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 305 310 315 320
 20 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 325 330 335
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 340 345 350
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 25 355 360 365
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375

 <210> 30
 30 <211> 152
 <212> PRT
 <213> Artificial sequence
 <400> 30
 Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly
 35 1 5 10 15
 Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe
 20 25 30

Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala
 35 40 45
 Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp
 50 55 60
 5 Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Val Pro Thr Asp
 65 70 75 80
 Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala
 85 90 95
 Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr
 10 100 105 110
 Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu
 115 120 125
 Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly
 130 135 140
 15 Ser His His His His His His His
 145 150
 <210> 31
 <211> 379
 <212> PRT
 20 <213> Artificial sequence
 <400> 31
 Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly
 1 5 10 15
 Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe
 25 20 25 30
 Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala
 35 40 45
 Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp
 50 55 60
 30 Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Ala Pro Ala Asn
 65 70 75 80
 Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala
 85 90 95
 Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr
 35 100 105 110
 Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu
 115 120 125

Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly
 130 135 140
 Ser Ala Ser Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 145 150 155 160
 5 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 165 170 175
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 180 185 190
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 10 195 200 205
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 210 215 220
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 225 230 235 240
 15 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 245 250 255

 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 260 265 270
 20 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 275 280 285
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 290 295 300
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 25 305 310 315 320
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 325 330 335
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 340 345 350
 30 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 355 360 365
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375

 35 <210> 32
 <211> 152
 <212> PRT

<213> Artificial sequence

<400> 32

Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly

1 5 10 15

5 Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe

20 25 30

Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala

35 40 45

Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp

10 50 55 60

Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Ala Pro Ala Asn

65 70 75 80

Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala

85 90 95

15 Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr

100 105 110

Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu

115 120 125

Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly

20 130 135 140

Ser His His His His His His His

145 150

<210> 33

25 <211> 1137

<212> DNA

<213> Artificial sequence

<400> 33

atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcagcta 60

30 ctatttaata aaacaaaatc tgtagaattc acgttttgta atgacactgt cgtcattcca 120

tgctttgta ctaatatgga ggcacaaaac actactgaag tatacgtaaa gtggaaattt 180

aaaggaagag atatttacac ctttgatgga gctctaaaca agtcactgt cccactgac 240

tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc ttgaagatg 300

gataagagtg atgtgtgtc acacacagga aactacactt gtgaagtaac agaattaacc 360

35 agagaagggtg aaacgatcat cgagctaaaa tctgtgttg ttcatgggtt ttctccatcc 420

ggaggtggag gtccgctag cgagcccaaa tctgtgaca aaactcacac atgccaccg 480

tgccagcac ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaaccaag 540

	gacaccctca tgalctcccg gaccctgag gtcacatgcg tgggtggtgga cgtgagccac	600
	gaagaccctg aggtcaagtt caactggtag gtggacggcg tggaggtgca taatgccaag	660
	acaaaagccgc gggaggagca glacaacagc acgtaccgtg tggtcagcgt cctcaccgtc	720
	ctgcaccagg actggctgaa tggcaaggag tacaagtga aggtctccaa caaagccctc	780
5	ccagccccc tgcagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg	840
	tacaccctgc ctccatctcg ggatgagctg accaagaacc aggtcagcct gacctgcctg	900
	gtcaaaggct tctatcccag cgacatgcc gtggagtggg agagcaatgg gcagccggag	960
	aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatagc	1020
	aagctcaccg tggacaagag caggtaggcag cagggggaacg tcttctcatg ctccgtgatg	1080
10	catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa	1137
	<210> 34	
	<211> 457	
	<212> DNA	
15	<213> Artificial sequence	
	<400> 34	
	atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcagcta	60
	ctatttaata aaacaaaatc tgtagaattc acgttttgta atgacactgt cgtcattcca	120
	tgcttttgta ctaatatgga ggcacaaaac actactgaag tatacgtaaa gtggaaattt	180
20	aaaggaagag atatttacac ctttgatgga gctctaaaca agtccactgt cccactgac	240
	tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc ttgaagatg	300
	gataagagtg atgtgtgtc acacacagga aactacactt gtgaagtaac agaattaacc	360
	agagaagggtg aaacgatcat cgagctaaaa tatcgtgttg ttcatgggtt ttcccatcc	420
	ggaggtggtg gatcccatca ccatcaccac catcatt	457
25	<210> 35	
	<211> 1137	
	<212> DNA	
	<213> Artificial sequence	
30	<400> 35	
	atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcaacta	60
	ctatttaata aaacaaaatc tgtagaattc acgttctgta atgacactgt cgtcattcca	120
	tgcttttgta ctaatatgga ggcacaaaac actaccgaag tatatgtaaa gtggaaattt	180
	aaaggaagag atatttacac gtttgatgga gctctaaaca agtccactgc ccccgtaac	240
35	tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc ttgaagatg	300
	gataagagtg atgtgtgtc acacacagga aactacactt gtgaagtaac agaattaacc	360
	agagaagggtg aaacgatcat tgagctaaaa tatcgtgttg ttctgtggtt ttcccatcc	420

ggaggtggag gttccgctag cgagcccaaa tcttgtgaca aaactcacac atgcccaccg 480
 tggccagcac ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaaccaag 540
 gacaccctca tgalctcccg gaccctgag gtcacatgcg tgggtggtgga cgtgagccac 600
 gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag 660
 5 acaaagccgc gggaggagca glacaacagc acgtaccgtg tggtcagcgt cctcaccgtc 720
 ctgcaccagg actggctgaa tggcaaggag tacaagtga aggtctccaa caaagccctc 780
 ccagccccca tcgagaaaac catctccaaa gccaaagggc agccccgaga accacagggtg 840
 tacaccctgc ctccatctcg ggatgagctg accaagaacc aggtcagcct gacctgcctg 900
 gtcaaagggt tctatcccag cgacatgcc gtggagtggg agagcaatgg gcagccggag 960
 10 aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatage 1020
 aagctcaccg tggacaagag caggtggcag cagggggaacg tcttctcatg ctccgtgatg 1080
 catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa 1137

<210> 36
 15 <211> 457
 <212> DNA
 <213> Artificial sequence
 <400> 36
 atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcaacta 60
 20 ctatttaata aaacaaaatc tgtagaattc acgttctgta atgacactgt cgtcattcca 120
 tgctttgtta ctaatatgga ggcacaaaac actaccgaag tatatgtaaa gtggaaattt 180
 aaaggaagag atatttacac gtttgatgga gctctaaaca agtccactgc ccccgctaac 240
 tttagtagtg caaaaattga agtctcaciaa ttactaaaag gagatgcctc tttgaagatg 300
 gataagagtg atgtgtctc acacacagga aactacactt gtgaagtaac agaattaacc 360
 25 agagaagggtg aaacgatcat tgagctaaaa tatcgtgttg ttctgtggtt ttctccatcc 420
 ggaggtggtg gatcccatca ccatcaccac catcatt 457

<210> 37
 <211> 444
 30 <212> PRT
 <213> Artificial sequence
 <400> 37
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 35 Ser Val Gln Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn His
 20 25 30
 Val Ile His Trp Leu Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

	35	40	45
	Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe		
	50	55	60
	Lys Asp Arg Val Thr Met Thr Ser Asp Thr Ser Ile Ser Thr Ala Tyr		
5	65	70	75
	Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys		
	85	90	95
	Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln Ala Thr Leu		
	100	105	110
10	Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu		
	115	120	125
	Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys		
	130	135	140
	Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser		
15	145	150	155
	Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser		
	165	170	175
	Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser		
	180	185	190
20	Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn		
	195	200	205
	Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro		
	210	215	220
	Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe		
25	225	230	235
	Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val		
	245	250	255
	Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe		
	260	265	270
30	Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro		
	275	280	285
	Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr		
	290	295	300
	Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val		
35	305	310	315
	Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala		
	325	330	335

	Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln	
	340 345 350	
	Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly	
	355 360 365	
5	Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro	
	370 375 380	
	Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser	
	385 390 395 400	
	Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu	
10	405 410 415	
	Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His	
	420 425 430	
	Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys	
	435 440	
15	<210> 38	
	<211> 1332	
	<212> DNA	
	<213> Artificial sequence	
20	<400> 38	
	cagggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgcaggtt	60
	tctgtgaagg catctggata caccctcacc aacctgtta ttactggct gcgacaggcc	120
	cctggacaag ggcttgagtg gatgggatattttatcctt acaatgatgg tactaagtat	180
	aatgagaagt tcaaggacag agtcacgatg acctcagaca cgtccatcag cacagcctac	240
25	atggaggtga gcagctcag atctgacgac acggccgtct attattgtgc tagagggggt	300
	tactatactt acgacgactg gggccaagca accctgggtca ccgtctcgag cgctagcacc	360
	aaggggccat ccgtcttccc cctggcgccc tgcctcagga gcacctcca gagcacagcc	420
	gccctggggt gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca	480
	ggcgccctga ccagcggcgt gcacaccttc ccggtgttc tacagtcctc aggactctac	540
30	tcctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacgaagac ctacacctgc	600
	aacgtagatc acaagcccag caacaccaag gtggacaaga gagttgagtc caaatatggt	660
	cccccatgcc caccatgccc agcacctgag ttctggggg gaccatcagt ctctctgttc	720
	cccccaaac ccaaggacac tctcatgac tcccggaccc ctgaggtcac gtgcgtggtg	780
	gtggacgtga gccaggaaga ccccgaggtc cagttcaact ggtacgtgga tggcgtggag	840
35	gtgcataatg ccaagacaaa gccgcgggag gagcagttca acagcacgta ccgtgtggtc	900
	agcgtctca ccgtctgca ccaggactgg ctgaacggca aggagtacaa gtgcaaggtc	960
	tccaacaaag gcctcccgtc ctccatcgag aaaacatct ccaaagccaa agggcagccc	1020

cgagagccac aggtgtacac cctgccccca tcccaggagg agatgaccaa gaaccaggtc 1080
 agcctgacct gcctggtaaa aggcctctac cccagcgaca tcgccgtgga gtgggagagc 1140
 aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc 1200
 ttcttctct acagcaggct aaccgtggac aagagcaggt ggcaggaggg gaatgtcttc 1260
 5 tcatgtcccg tgatgcatga ggctctgcac aaccactaca cacagaagag cctctccctg 1320
 tctctgggta aa 1332

<210> 39
 <211> 219
 10 <212> PRT
 <213> Artificial sequence
 <400> 39
 Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15
 15 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30
 Asn Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 20 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 Asp Asp Val Gly Ile Tyr Tyr Cys Ser Gln Ser
 25 85 90 95
 Thr His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 30 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 35 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215
 5
 <210> 40
 <211> 657
 <212> PRT
 <213> Artificial sequence
 10
 <400> 40
 Gly Ala Thr Ala Thr Thr Gly Thr Gly Ala Thr Gly Ala Cys Thr Cys
 1 5 10 15
 Ala Gly Thr Cys Thr Cys Cys Ala Cys Thr Cys Thr Cys Cys Cys Thr
 20 25 30
 15
 Gly Cys Cys Cys Gly Thr Cys Ala Cys Cys Cys Cys Thr Gly Gly Ala
 35 40 45
 Gly Ala Gly Cys Cys Gly Gly Cys Cys Thr Cys Cys Ala Thr Cys Thr
 50 55 60
 Cys Cys Thr Gly Cys Ala Gly Ala Thr Cys Ala Ala Gly Thr Cys Ala
 20 65 70 75 80
 Gly Ala Gly Cys Cys Thr Thr Gly Thr Gly Cys Ala Cys Ala Gly Thr
 85 90 95
 Ala Ala Thr Gly Gly Ala Ala Ala Gly Ala Cys Cys Thr Ala Thr Thr
 100 105 110
 25
 Thr Ala Cys Ala Thr Thr Gly Gly Thr Ala Thr Cys Thr Gly Cys Ala
 115 120 125
 Gly Ala Ala Gly Cys Cys Ala Gly Gly Cys Cys Ala Gly Thr Cys Thr
 130 135 140
 Cys Cys Ala Ala Gly Ala Cys Thr Cys Cys Thr Gly Ala Thr Cys Thr
 30 145 150 155 160
 Ala Cys Ala Ala Ala Gly Thr Thr Thr Cys Cys Ala Ala Cys Cys Gly
 165 170 175
 Ala Thr Thr Thr Thr Cys Thr Gly Gly Thr Gly Thr Cys Cys Cys Ala
 180 185 190
 35
 Gly Ala Cys Ala Gly Ala Thr Thr Cys Ala Gly Cys Gly Gly Cys Ala
 195 200 205
 Gly Thr Gly Gly Gly Thr Cys Ala Gly Gly Cys Ala Cys Thr Gly Ala

	210	215	220
	Thr Thr Thr Cys Ala Cys Ala Cys Thr Gly Ala Ala Ala Thr Cys		
	225	230	235
	Ala Gly Cys Ala Gly Gly Gly Thr Gly Gly Ala Gly Gly Cys Thr Gly		240
5	245	250	255
	Ala Thr Gly Ala Thr Gly Thr Thr Gly Gly Ala Ala Thr Thr Thr Ala		
	260	265	270
	Thr Thr Ala Cys Thr Gly Cys Thr Cys Thr Cys Ala Ala Ala Gly Thr		
	275	280	285
10	Ala Cys Ala Cys Ala Thr Gly Thr Thr Cys Cys Gly Thr Ala Cys Ala		
	290	295	300
	Cys Gly Thr Thr Thr Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys		
	305	310	315
	Cys Ala Ala Gly Cys Thr Gly Gly Ala Gly Ala Thr Cys Ala Ala Ala		320
15	325	330	335
	Cys Gly Gly Ala Cys Cys Gly Thr Gly Gly Cys Gly Gly Cys Gly Cys		
	340	345	350
	Cys Ala Thr Cys Thr Gly Thr Cys Thr Thr Cys Ala Thr Cys Thr Thr		
	355	360	365
20	Cys Cys Cys Gly Cys Cys Ala Thr Cys Thr Gly Ala Thr Gly Ala Gly		
	370	375	380
	Cys Ala Gly Thr Thr Gly Ala Ala Ala Thr Cys Thr Gly Gly Thr Ala		
	385	390	395
	Cys Cys Gly Cys Thr Ala Gly Cys Gly Thr Thr Gly Thr Gly Thr Gly		400
25	405	410	415
	Cys Cys Thr Gly Cys Thr Gly Ala Ala Thr Ala Ala Cys Thr Thr Cys		
	420	425	430
	Thr Ala Thr Cys Cys Cys Ala Gly Ala Gly Ala Gly Gly Cys Cys Ala		
	435	440	445
30	Ala Ala Gly Thr Ala Cys Ala Gly Thr Gly Gly Ala Ala Gly Gly Thr		
	450	455	460
	Gly Gly Ala Thr Ala Ala Cys Gly Cys Cys Cys Thr Cys Cys Ala Ala		
	465	470	475
	Thr Cys Gly Gly Gly Thr Ala Ala Cys Thr Cys Cys Cys Ala Gly Gly		
35	485	490	495
	Ala Gly Ala Gly Thr Gly Thr Cys Ala Cys Ala Gly Ala Gly Cys Ala		
	500	505	510

	Gly Gly Ala Cys Ala Gly Cys Ala Ala Gly Gly Ala Cys Ala Gly Cys	
	515 520 525	
	Ala Cys Cys Thr Ala Cys Ala Gly Cys Cys Thr Cys Ala Gly Cys Ala	
	530 535 540	
5	Gly Cys Ala Cys Cys Cys Thr Gly Ala Cys Gly Cys Thr Gly Ala Gly	
	545 550 555 560	
	Cys Ala Ala Ala Gly Cys Ala Gly Ala Cys Thr Ala Cys Gly Ala Gly	
	565 570 575	
	Ala Ala Ala Cys Ala Cys Ala Ala Ala Gly Thr Cys Thr Ala Cys Gly	
10	580 585 590	
	Cys Cys Thr Gly Cys Gly Ala Ala Gly Thr Cys Ala Cys Cys Cys Ala	
	595 600 605	
	Thr Cys Ala Gly Gly Gly Cys Cys Thr Gly Ala Gly Cys Thr Cys Gly	
	610 615 620	
15	Cys Cys Cys Gly Thr Cys Ala Cys Ala Ala Ala Gly Ala Gly Cys Thr	
	625 630 635 640	
	Thr Cys Ala Ala Cys Ala Gly Gly Gly Gly Ala Gly Ala Gly Thr Gly	
	645 650 655	
	Thr	
20		
	<210> 41	
	<211> 21	
	<212> DNA	
25	<213> Artificial sequence	
	<400> 41	
	ctcaggggaar tarccyttga c	21
	<210> 42	
30	<211> 21	
	<212> DNA	
	<213> Artificial sequence	
	<400> 42	
	tcactgccat caatcttcca c	21
35		
	<210> 43	
	<211> 18	

<212> DNA
 <213> Artificial sequence
 <400> 43
 tgtaaaacga cggccagt 18
 5
 <210> 44
 <211> 18
 <212> DNA
 <213> Artificial sequence
 10 <400> 44
 caggaaacag ctatgacc 18
 <210> 45
 <211> 5
 15 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(5)
 20 <223> Xaa (3) =Val、Phe、Leu; Xaa (4) =Met、Val、Trp、Leu
 <400> 45
 Asn Tyr Xaa Xaa His
 1 5
 <210> 46
 25 <211> 17
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 30 <222> (1)..(17)
 <223> Xaa (10) =Asn、Lys、Cys; Xaa (12) =Asn、Ser、Ala
 <400> 46
 Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Xaa Tyr Xaa Glu Lys Phe Lys
 1 5 10 15
 35 Gly
 <210> 47

<211> 8
 <212> PRT
 <213> Artificial sequence
 <220>
 5 <221> MOD_RES
 <222> (1)..(8)
 <223> Xaa (3) =Trp、Lys、Thr、Leu; Xaa (4) =Ser、Thr、Lys; Xaa (5) =Met、
 Phe、Gly
 <400> 47
 10 Gly Gly Xaa Phe Xaa Xaa Asp Tyr
 1 5

 <210> 48
 <211> 5
 15 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(5)
 20 <223> Xaa (3) =Glu、Gly、Thr
 <400> 48
 Asp Tyr Xaa Met His
 1 5

 25 <210> 49
 <211> 17
 <212> PRT
 <213> Artificial sequence
 <220>
 30 <221> MOD_RES
 <222> (1)..(17)
 <223> Xaa (5) =Glu、Tyr、Lys; Xaa (8) =Gly、Asn、Pro; Xaa (13) =Glu、
 Asn、Gly、Ser
 <400> 49
 35 Ala Ile Asp Pro Xaa Thr Gly Xaa Thr Ala Tyr Asn Xaa Lys Phe Lys
 1 5 10 15
 Gly

5 <210> 50
 <211> 8
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(8)
 <223> Xaa (4) =Tyr、Lys、Leu
 10 <400> 50
 Gly Thr Pro Xaa Ala Met Asp Tyr
 1 5

15 <210> 51
 <211> 5
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 20 <222> (1)..(5)
 <223> Xaa (3) =Phe、Asn、Tys; Xaa (4) =Leu、Asn、Gly
 <400> 51
 Asn Tyr Xaa Xaa His
 1 5

25 <210> 52
 <211> 17
 <212> PRT
 <213> Artificial sequence
 30 <220>
 <221> MOD_RES
 <222> (1)..(17)
 <223> Xaa (4) =Pro、Gly、Ser、Cys、Lys; Xaa (14) =Asn、Lys
 <400> 52
 35 Tyr Ile Asn Xaa Tyr Asn Asp Gly Thr Asn Tyr Asn Glu Xaa Phe Lys
 1 5 10 15
 Gly

5 <210> 53
 <211> 10
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(10)
 10 <223> Xaa (2) =Lys, Trp, Phe; Xaa (7) =Ser, Asp, Tyr
 <400> 53
 Ala Xaa Gly Gly Trp Phe Xaa Met Asp Tyr
 1 5 10

15 <210> 54
 <211> 16
 <212> PRT
 <213> Artificial sequence
 <220>
 20 <221> MOD_RES
 <222> (1)..(16)
 <223> Xaa (10) =Asn, Lys, Gly; Xaa (12) =Lys, Gly, Asn; Xaa (16) =Glu,
 His, Cys, Lys
 <400> 54
 25 Arg Ser Ser Gln Ser Leu Val His Ser Xaa Gly Xaa Thr Tyr Leu Xaa
 1 5 10 15

30 <210> 55
 <211> 7
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(7)
 35 <223> Xaa (1) =Lys, Arg
 <400> 55
 Xaa Val Ser Asn Arg Phe Ser

1 5

<210> 56
 <211> 8
 5 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(8)
 10 <223> Xaa (7) =Tyr、Pro、Phe、Asp
 <400> 56
 Ser Gln Ser Thr His Val Xaa Thr
 1 5

15 <210> 57
 <211> 16
 <212> PRT
 <213> Artificial sequence
 <220>
 20 <221> MOD_RES
 <222> (1)..(16)
 <223> Xaa (4) =Tyr、Pro、Phe、Asp; Xaa (11) =Gly、Lys、Asn、Ser
 <400> 57
 Arg Ser Ser Xaa Ser Leu Val His Ser Asn Xaa Asn Thr Tyr Leu His
 25 1 5 10 15
 <210> 58
 <211> 7
 <212> PRT
 <213> Artificial sequence
 30 <220>
 <221> MOD_RES
 <222> (1)..(7)
 <223> Xaa (5) =Arg、Gly、Ser、Lys
 <400> 58
 35 Lys Val Ser Asn Xaa Phe Ser
 1 5

<210> 59
 <211> 9
 <212> PRT
 <213> Artificial sequence
 5 <220>
 <221> MOD_RES
 <222> (1)..(9)
 <223> Xaa (4) =Thr、 Lys
 <400> 59
 10 Ser Gln Ser Xaa His Val Pro Trp Thr
 1 5

 <210> 60
 <211> 16
 15 <212> PRT
 <213> Artificial sequence
 <220>
 <221> MOD_RES
 <222> (1)..(16)
 20 <223> Xaa (6) =Leu、 Arg; Xaa (14) =Tyr、 Lys、 Leu、 Gly
 <400> 60
 Arg Ser Ser Gln Ser Xaa Val His Ser Asn Gly Lys Thr Xaa Leu Gln
 1 5 10 15

 25 <210> 61
 <211> 7
 <212> PRT
 <213> Artificial sequence
 <220>
 30 <221> MOD_RES
 <222> (1)..(7)
 <223> Xaa (2) =Val、 Asn、 Lys、 Ser
 <400> 61
 Lys Xaa Ser Asn Arg Phe Ser
 35 1 5

 <210> 62

<211> 10
 <212> PRT
 <213> Artificial sequence
 <220>
 5 <221> MOD_RES
 <222> (1)..(8)
 <223> Xaa (2) =Gln、Cys; Xaa (7) =Tys、Pro、Trp、Asp
 <400> 62
 Ser Xaa Gln Ser Thr His Val Xaa Tyr Thr
 10 1 5 10

What is claimed is:

1. An isolated antigen comprising the amino acid sequence according to any one of SEQ ID NOs: 29 to 32.

2. An anti-CD47 monoclonal antibody, comprising:

(I) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 1, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 8;

(II) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 2, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 9;

(III) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 3, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 10;

(IV) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 4, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 11;

(V) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 5, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 12;

(VI) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 6, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 13; and

(VII) a heavy chain variable region with an amino acid sequence represented by SEQ ID NO: 7, and a light chain variable region with an amino acid sequence represented by SEQ ID NO: 14.

3. The anti-CD47 monoclonal antibody according to claim 2, wherein the heavy chain type is IgG1, IgG3 or IgM; and the light chain type is κ .

4. A nucleotide sequence or sequences encoding the anti-CD47 monoclonal antibody according to claim 2 or claim 3.

5. The nucleotide sequence or sequences according to claim 4, comprising:

(I) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 15, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 22; or

5 (II) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 16, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 23; or

(III) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 17, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 24; or

10 (IV) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 18, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 25; or

15 (V) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 19, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 26; or

(VI) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 20, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 27; or

20 (VII) a nucleotide sequence of the heavy chain variable region as represented by SEQ ID NO: 21, and a nucleotide sequence of the light chain variable region as represented by SEQ ID NO: 28; or

(VIII) a sequence or sequences complementary with any one of (I) to (VII); or

25 (IX) a nucleotide sequence which encodes the same protein as the nucleotide sequence of any one of (I) to (VIII) but differs from the nucleotide sequence of any one of (I) or (VIII) due to the degeneracy of the genetic code.

6. An expression vector or vectors comprising the nucleotide sequence or sequences according to claim 4 or claim 5.

7. A host cell transformed with the expression vector or vectors according to claim 6.

30 8. A hybridoma cell strain, which produces the anti-CD47 monoclonal antibody according to claim 2 or claim 3.

9. A method for preparing the anti-CD47 monoclonal antibody according to claim 2 or claim 3, wherein the method comprises:

step 1: after immunizing a mouse with the antigen according to claim 1, obtaining spleen cells of the mouse;

step 2: fusing the spleen cells with myeloma cells, screening for a hybridoma cell strain capable of binding to CD47, and culturing the cell strain *in vitro* to obtain the anti-CD47 monoclonal antibody.

10. A combination, which is prepared by marking the anti-CD47 monoclonal antibody according to claim 2 or claim 3 with a chemical or biological marker.

11. The combination according to claim 10, wherein the chemical marker is an isotope, an immunotoxin and/or a chemical drug, and the biological marker is biotin, avidin or an enzyme label.

12. A conjugate, which is prepared by coupling the anti-CD47 monoclonal antibody according to claim 2 or claim 3 or the combination according to claim 10 or claim 11 to a solid medium or a semi-solid medium.

13. Use of the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to claim 12 in the preparation of a product for detecting expression of CD47.

14. A kit comprising the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to claim 12.

15. Use of the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to claim 12 in the preparation of a formulation for blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells.

16. A method of blocking binding between CD47 and SIRP α and/or for increasing macrophage phagocytic index against tumor cells and/or for promoting apoptosis of tumor cells, comprising administering the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to

claim 12.

17. The use according to claim 15, or the method according to claim 16, wherein the tumor cells are human peripheral blood leukemia T cells.

18. Use of the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to claim 12 in the preparation of a medicament for preventing or treating a disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma.

19. A medicament comprising the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, and/or the conjugate according to claim 12.

20. A method for preventing or treating a disease, wherein the disease is leukemia, lymphoma, breast cancer, lung cancer, gastric cancer, intestinal cancer, esophageal cancer, ovarian cancer, cervical cancer, kidney cancer, bladder cancer, pancreatic cancer, glioma and/or melanoma, comprising administering the anti-CD47 monoclonal antibody according to claim 2 or claim 3, the combination according to claim 10 or claim 11, the conjugate according to claim 12, and/or the medicament according to claim 19.

Figure 1

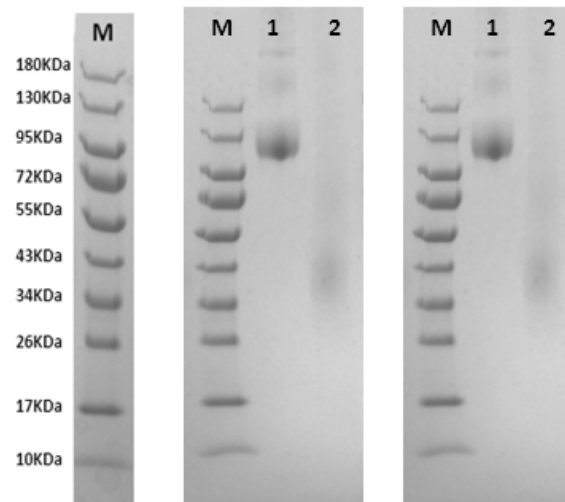


Figure 1-A Figure 1-B

Figure 2

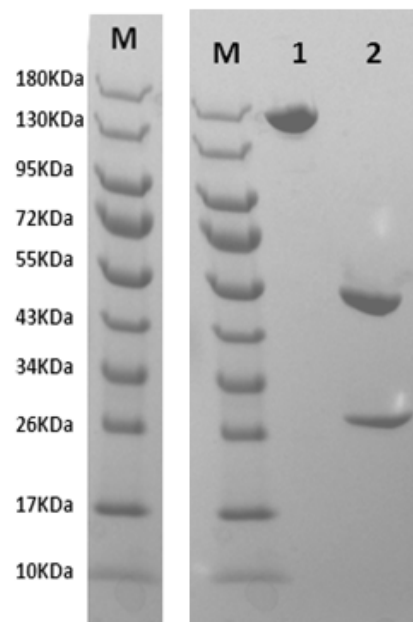


Figure 3

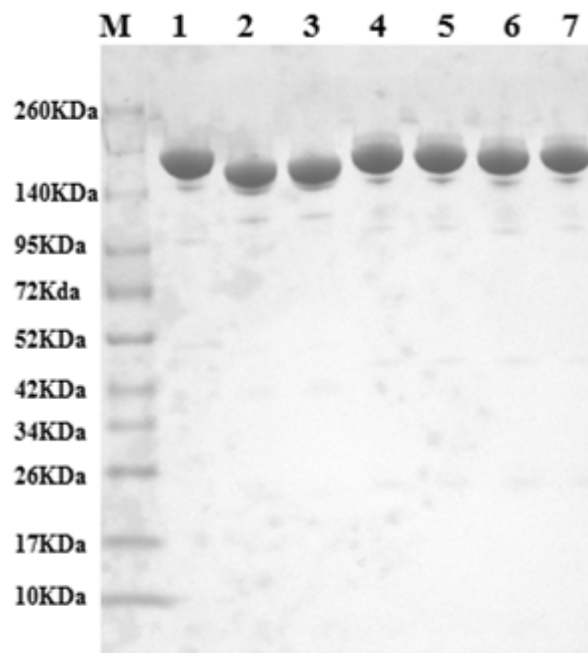


Figure 4

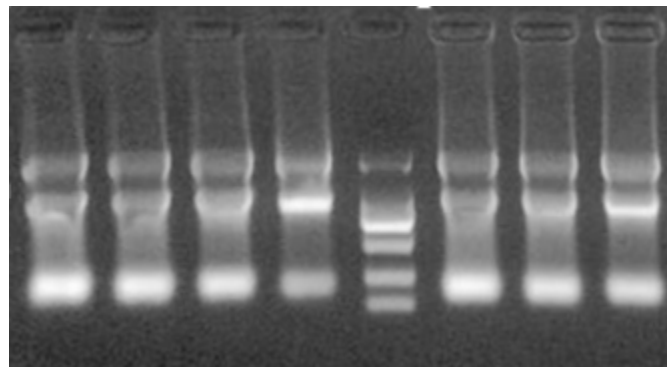


Figure 5

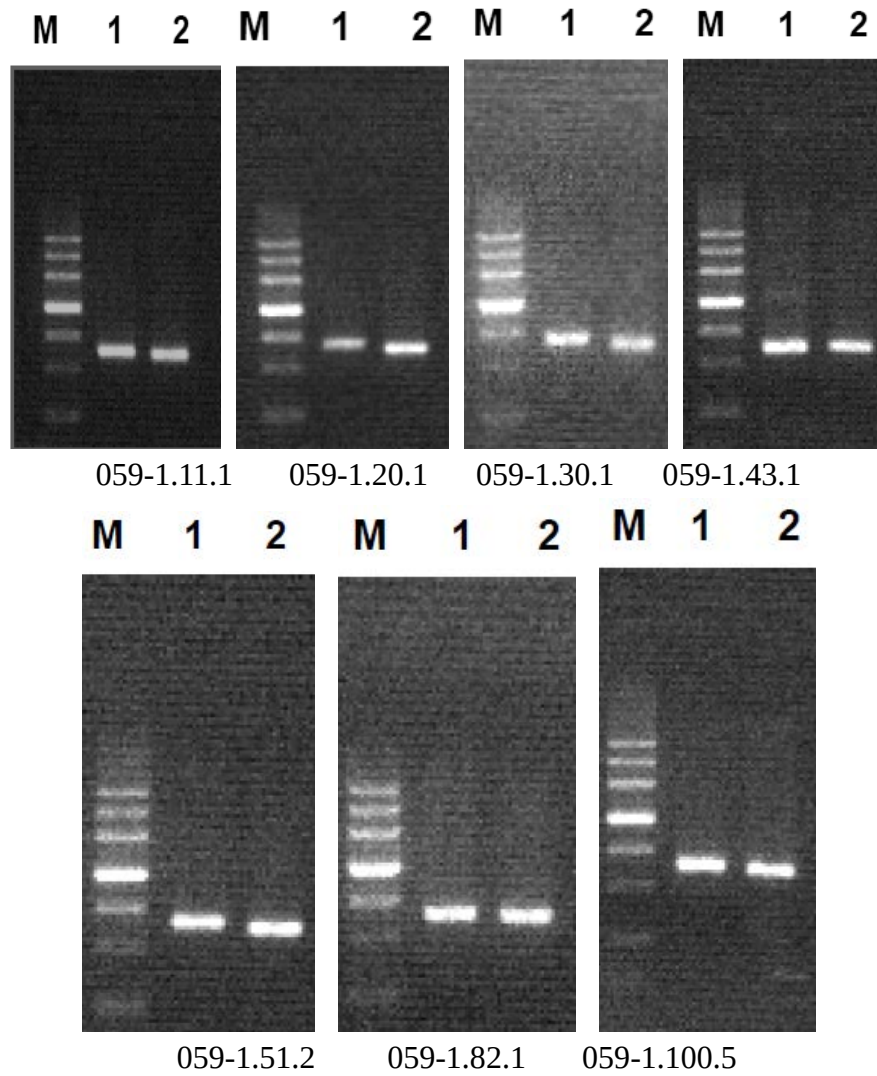


Figure 6

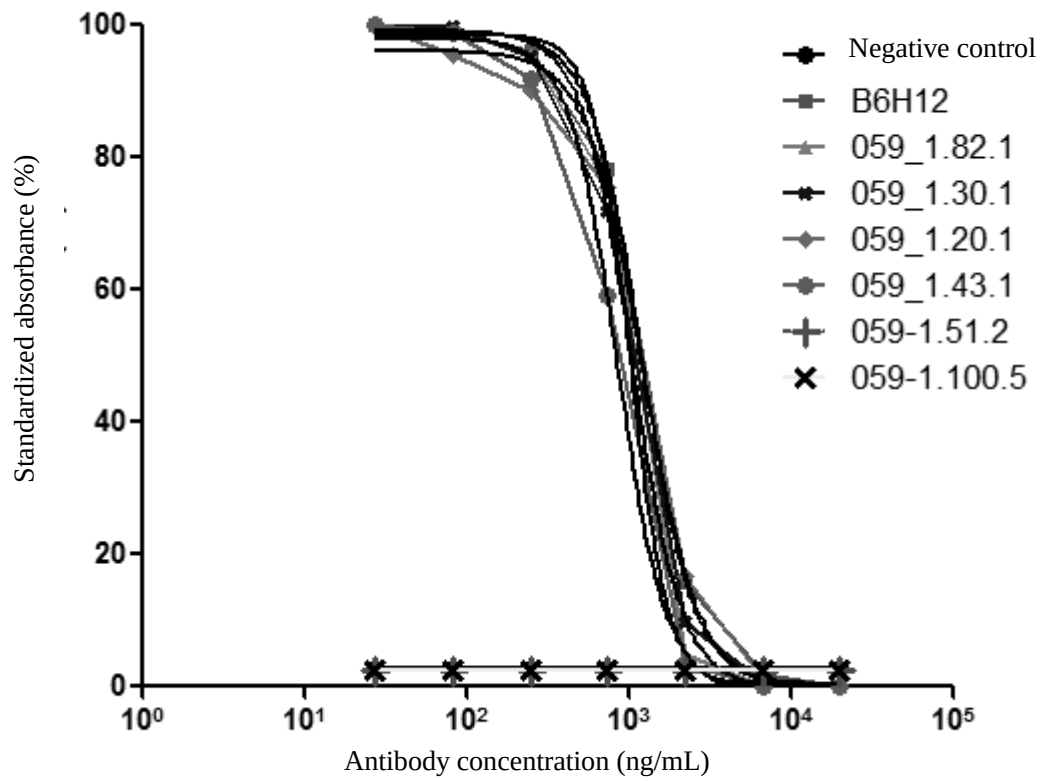


Figure 7

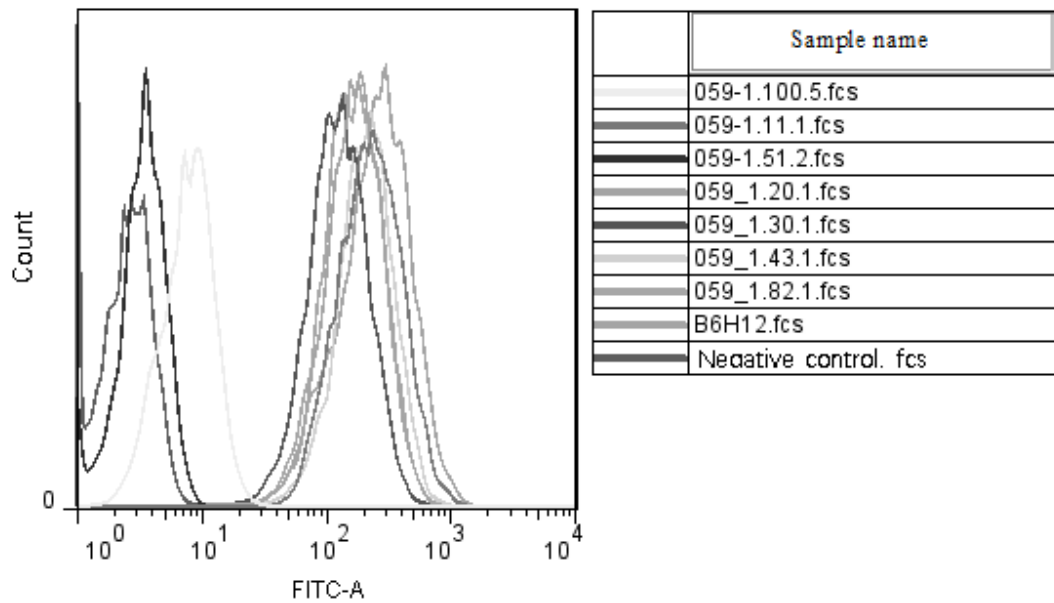
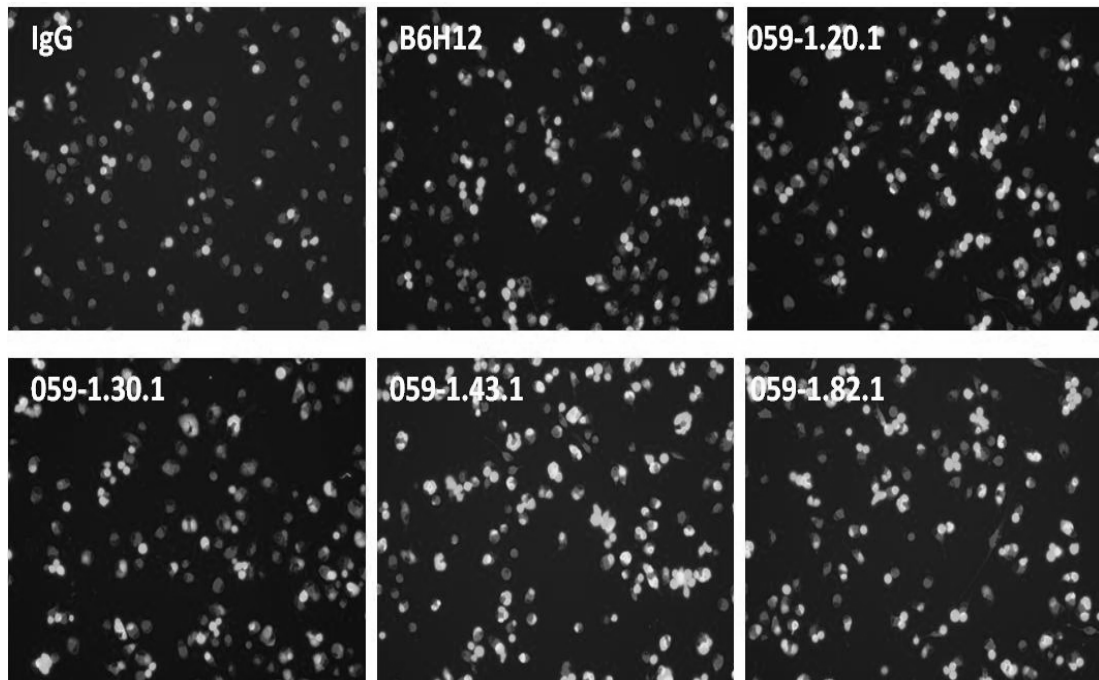


Figure 8



SEQUENCE LISTING

<110> CHANGCHUN GENESCENCE PHARMACEUTICALS CORP.
 <120> Anti-CD47 monoclonal antibody and application thereof
 <130> OP170553
 <160> 62
 <170> PatentIn version 3.3

<210> 1
 <211> 136
 <212> PRT
 <213> Artificial sequence
 <400> 1
 Met Glu Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Val Ile Ala Gly
 1 5 10 15
 Val Gln Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
 20 25 30
 Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr Asp Tyr Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu
 50 55 60
 Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn
 65 70 75 80
 Gln Asn Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
 85 90 95
 Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110
 Tyr Tyr Cys Thr Arg Gly Thr Pro Tyr Ala Met Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser
 130 135

<210> 2
 <211> 136
 <212> PRT
 <213> Artificial sequence
 <400> 2
 Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30
 Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr Asn Tyr Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
 50 55 60
 Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Asn Tyr Asn
 65 70 75 80
 Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser
 85 90 95
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110
 Tyr Tyr Cys Ala Lys Gly Gly Trp Phe Ser Met Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser
 130 135

```

<210> 3
<211> 136
<212> PRT
<213> Artificial sequence
<400> 3
Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1          5          10          15
Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
20          25          30
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35          40          45
Thr Asn Tyr Val Met His Trp Met Lys Gln Lys Pro Gly Gln Gly Leu
50          55          60
Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Asp Thr Lys Tyr Asn
65          70          75          80
Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser
85          90          95
Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
100         105         110
Tyr Tyr Cys Ala Lys Gly Gly Tyr Tyr Ser Met Asp Tyr Trp Gly Gln
115         120         125
Gly Thr Ser Val Thr Val Ser Ser
130         135

```

```

<210> 4
<211> 136
<212> PRT
<213> Artificial sequence
<400> 4
Met Glu Trp Ser Trp Val Phe Leu Phe Leu Leu Ser Val Ile Ala Gly
1          5          10          15
Val Gln Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
20          25          30
Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35          40          45
Thr Asp Tyr Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu
50          55          60
Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn
65          70          75          80
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
85          90          95
Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
100         105         110
Tyr Tyr Cys Thr Arg Gly Thr Pro Tyr Ala Met Asp Tyr Trp Gly Gln
115         120         125
Gly Thr Ser Val Thr Val Ser Ser
130         135

```

```

<210> 5
<211> 136
<212> PRT
<213> Artificial sequence
<400> 5
Met Glu Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1          5          10          15
Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys

```

			20					25					30				
Pro	Gly	Ala	Ser	Val	Arg	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe		
		35					40					45					
Thr	Asn	Tyr	Val	Met	His	Trp	Val	Lys	Gln	Lys	Pro	Gly	Gln	Gly	Leu		
	50					55					60						
Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro	Tyr	Asn	Asp	Gly	Thr	Lys	Tyr	Ser		
65					70					75					80		
Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ser	Asp	Lys	Ser	Ser	Ile		
			85						90					95			
Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val		
			100					105						110			
Tyr	Tyr	Cys	Ala	Lys	Gly	Gly	Leu	Phe	Thr	Phe	Asp	Tyr	Trp	Gly	Gln		
		115					120					125					
Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser										
	130					135											

<210> 6

<211> 136

<212> PRT

<213> Artificial sequence

<400> 6

Met	Glu	Trp	Ser	Trp	Ile	Phe	Leu	Phe	Leu	Leu	Ser	Gly	Thr	Ala	Gly		
1				5				10						15			
Val	His	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys		
			20					25					30				
Pro	Gly	Ala	Ser	Val	Asn	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe		
		35					40					45					
Thr	Asn	Tyr	Phe	Leu	His	Trp	Val	Lys	Gln	Lys	Pro	Gly	Gln	Gly	Leu		
	50					55					60						
Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro	Tyr	Asn	Asp	Gly	Thr	Asn	Tyr	Asn		
65					70					75					80		
Glu	Asn	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ser	Asp	Lys	Ser	Ser	Ser		
			85						90					95			
Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val		
			100					105						110			
Tyr	Tyr	Cys	Ala	Lys	Gly	Gly	Trp	Phe	Ser	Met	Asp	Tyr	Trp	Gly	Gln		
		115					120					125					
Gly	Thr	Ser	Val	Thr	Val	Ser	Ser										
	130					135											

<210> 7

<211> 136

<212> PRT

<213> Artificial sequence

<400> 7

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

2017284157

Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser
				85					90					95	
Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val
			100					105					110		
Tyr	Tyr	Cys	Thr	Arg	Gly	Thr	Pro	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln
		115					120					125			
Gly	Thr	Ser	Val	Thr	Val	Ser	Ser								
	130					135									

$\langle 210 \rangle$ 8

$\langle 211 \rangle$ 131

<212> PRT

<213> Artificial sequence

<400>	8
-------	---

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1 5 10 15

Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
35 40 45

Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
100 105 110

Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu

Glu Ile Lys
130

<210> 9

$\langle 211 \rangle$ 130

<212> PRT

<213> Artificial sequence

$\langle 400 \rangle$	9
-----------------------	---

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1 5 10 15

Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu

Val His Ser Asn Gly Lys Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
100 105 110

Ser Gln Ser Thr His Val Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu

Ile Lys

130

<210> 10

<211> 131

<212> PRT

<213> Artificial sequence

<400> 10

```

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1          5          10          15
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
          20          25          30
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
          35          40          45
Val His Ser Lys Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
          50          55          60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65          70          75          80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
          85          90          95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
          100          105          110
Ser Gln Ser Thr His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
          115          120          125
Glu Ile Lys
          130

```

<210> 11

<211> 131

<212> PRT

<213> Artificial sequence

<400> 11

```

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1          5          10          15
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
          20          25          30
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
          35          40          45
Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
          50          55          60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65          70          75          80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
          85          90          95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
          100          105          110
Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
          115          120          125
Glu Ile Lys
          130

```

<210> 12

<211> 131

<212> PRT

<213> Artificial sequence

<400> 12

```

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala

```

```

1      5      10      15
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
      20      25      30
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
      35      40      45
Val His Ser Lys Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
      50      55      60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser
65      70      75      80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
      85      90      95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
      100      105      110
Ser Gln Ser Thr His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
      115      120      125
Glu Ile Lys
      130

```

```

<210> 13
<211> 130
<212> PRT
<213> Artificial sequence
<400> 13

```

```

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1      5      10      15
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
      20      25      30
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
      35      40      45
Val His Ser Asn Gly Lys Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro
      50      55      60
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65      70      75      80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
      85      90      95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
      100      105      110
Ser Gln Ser Thr His Val Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
      115      120      125
Ile Lys
      130

```

```

<210> 14
<211> 131
<212> PRT
<213> Artificial sequence
<400> 14

```

```

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

```

11 Jan 2019

2017284157

65	Gly	Val	Pro	Glu	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr
					85					90					95	
	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Phe	Cys
			100						105					110		
	Ser	Gln	Ser	Thr	His	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu
			115					120					125			
	Glu	Ile	Lys													
			130													

```

<210> 15
<211> 408
<212> DNA
<213> Artificial sequence
<400> 15
atggaatgga gctgggtcctt tctcttcctc ctgtcagtaa ttgcaggtgt ccaatcccag
60
gttcaactgc agcagtctgg ggctgagctg gtgaggcctg gggcttcagt gacgctgtcc
120
tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct
180
gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggtggtac tgcctacaat
240
cagaacttca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg
300
gagctccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc
360
tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

```

```

<210> 16
<211> 408
<212> DNA
<213> Artificial sequence
<400> 16
atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag
60
gtccagctgc agcagtctgg acctgagctg gtaaagcctg gggcttcagt gaagatgtcc
120
tgcaaggctt ctggatacac attcactaac tatgttatgc actgggtgaa gcagaagcct
180
ggacagggcc ttgagtggat tggatatatt aatccttaca atgatggtac taactacaat
240
gagaagttca aaggcaaggc cacactgact tcagacaaat cctccagcac agcctacatg
300
gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa aggggggtgg
360
ttctccatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

```

```

<210> 17
<211> 408
<212> DNA
<213> Artificial sequence
<400> 17

```

atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag
60
gtccagctgc agcagtctgg acctgagctg gtaaagcctg gggcttcagt gaagatgtcc
120
tgcaaggctt ctggatacac attcactaac tatgttatgc actggatgaa gcagaagcct
180
gggcagggcc ttgagtggat tggatatatt aatccttaca atgatgatac taaatacaat
240
gagaaattca aagacagggc cacactgact tcagacaaat cctccagcac agcctacatg
300
gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa ggggggttac
360
tattctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

<210> 18
<211> 408
<212> DNA
<213> Artificial sequence
<400> 18

atggaatgga gctgggtctt tctcttcctc ctgtcagtaa ttgcaggtgt ccaatcccag
60
gttcaactgc agcagtctgg ggctgagctg gtgaggcctg gggcttcagt gacgctgtcc
120
tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct
180
gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggtggtac tgcctacaat
240
cagaagttca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg
300
gaactccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc
360
tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

<210> 19
<211> 408
<212> DNA
<213> Artificial sequence
<400> 19

atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag
60
gtccagctgc agcagtctgg acctgagctg gtaaagcctg gggcttcagt gaggatgtcc
120
tgcaaggctt ctggatacac attcactaac tatgttatgc actgggtgaa gcagaagcct
180
gggcagggac ttgagtggat tggatatatt aatccttaca atgatggtac taagtacagt
240
gagaagttca aaggcaaggc cacactgact tcagacaaat cctccatcac agcctatatg
300
gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa aggggggctt
360
tttaccttg actactgggg ccaaggcacc actctcacag tctcctca
408

<210> 20

```

<211> 408
<212> DNA
<213> Artificial sequence
<400> 20
atggaatgga gttggatatt tctctttctc ctgtcaggaa ctgcaggtgt ccactctgag
60
gtccagctgc agcagtctgg acctgagctg gtaaagcctg gggcttcagt taatatgtcc
120
tgcaaggctt ctggatacac attcactaac tattttttgc actgggtgaa gcagaagcct
180
gggcagggcc ttgagtggat tggatatatt aatccttaca atgatggtag taactacaat
240
gagaacttca aaggcaaggc cactctgact tcagacaaat cctccagcac agcctacatg
300
gagctcagca gcctgacctc tgaggactct gcggtctatt actgtgcaaa aggggggtgg
360
ttctccatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

```

```

<210> 21
<211> 408
<212> DNA
<213> Artificial sequence
<400> 21
atggaatgga gctgggtctt tctcttcctc ctgtcagtaa ttgcaggtgt ccaatcccag
60
gttcaactgc agcagtctgg ggctgagctg gtgaggcctg gggcttcagt gacgctgtcc
120
tgcaaggctt cgggctacac atttactgac tatgaaatgc actgggtgaa gcagacacct
180
gtgcatggcc tggaatggat tggagctatt gatcctgaaa ctggttggtac tgcctacaat
240
cagaagttca agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg
300
gagctccgca gcctgacatc tgaggactct gccgtctatt actgtacaag aggtaccccc
360
tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca
408

```

```

<210> 22
<211> 393
<212> DNA
<213> Artificial sequence
<400> 22
atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcttgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ctttgtacac agtaatggaa acacctatct acattggtag
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcaggacag atttcacact caagatcagc
300
agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccgtgg
360

```

acgttcggtg gaggcaccaa gctggaaatc aaa 393

<210> 23
<211> 390
<212> DNA
<213> Artificial sequence
<400> 23

atgaagttgc ctgtaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaatggaa aaacctatct acagtggatc
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
300
agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgtttacacg
360
ttcggagggg ggaccaagct ggaaataaaa 390

<210> 24
<211> 393
<212> DNA
<213> Artificial sequence
<400> 24

atgaagttgc ctgtaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actttctctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaaaggaa acacctatct acattggatc
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
300
agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccattc
360
acgttcggtc cggggacaaa gttggaaata aaa 393

<210> 25
<211> 393
<212> DNA
<213> Artificial sequence
<400> 25

atgaagttgc ctgtaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaatggaa acacctatct acattggatc
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
300

agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccgtgg
360
acgttcggtg gaggcaccaa gctggaaatc aaa 393

<210> 26
<211> 393
<212> DNA
<213> Artificial sequence
<400> 26

atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagcc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaaaggaa acacctatctt acattggtac
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca gagtgtccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
300
agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccattc
360
acgttcggct cggggacaaa gttggaaata aaa 393

<210> 27
<211> 390
<212> DNA
<213> Artificial sequence
<400> 27

atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaatggaa aaacctatctt acagtgggtac
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240
ggggtcccag acaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
300
agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgtttacacg
360
ttcggagggg ggaccaagct ggaaataaaa 390

<210> 28
<211> 393
<212> DNA
<213> Artificial sequence
<400> 28

atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat
60
gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc
120
tcttgcagat ctagtcagag ccttgtacac agtaatggaa acacctatctt acattggtac
180
ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct
240

ggggtcccag aaaggttcag tggcagtgga tcagggacag atttcacact caagatcagc
 300
 agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccgtgg
 360
 acgttcggtg gaggcaccaa gctggaaatc aaa 393

<210> 29
 <211> 379
 <212> PRT
 <213> Artificial sequence
 <400> 29

Met	Trp	Pro	Leu	Val	Ala	Ala	Leu	Leu	Leu	Gly	Ser	Ala	Cys	Cys	Gly
1			5					10					15		
Ser	Ala	Gln	Leu	Phe	Asn	Lys	Thr	Lys	Ser	Val	Glu	Phe	Thr	Phe	
		20					25					30			
Cys	Asn	Asp	Thr	Val	Val	Ile	Pro	Cys	Phe	Val	Thr	Asn	Met	Glu	Ala
		35					40					45			
Gln	Asn	Thr	Thr	Glu	Val	Tyr	Val	Lys	Trp	Lys	Phe	Lys	Gly	Arg	Asp
	50					55					60				
Ile	Tyr	Thr	Phe	Asp	Gly	Ala	Leu	Asn	Lys	Ser	Thr	Val	Pro	Thr	Asp
65					70					75					80
Phe	Ser	Ser	Ala	Lys	Ile	Glu	Val	Ser	Gln	Leu	Leu	Lys	Gly	Asp	Ala
				85					90					95	
Ser	Leu	Lys	Met	Asp	Lys	Ser	Asp	Ala	Val	Ser	His	Thr	Gly	Asn	Tyr
			100					105					110		
Thr	Cys	Glu	Val	Thr	Glu	Leu	Thr	Arg	Glu	Gly	Glu	Thr	Ile	Ile	Glu
		115					120					125			
Leu	Lys	Tyr	Arg	Val	Val	Ser	Trp	Phe	Ser	Pro	Ser	Gly	Gly	Gly	Gly
	130					135					140				
Ser	Ala	Ser	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro
145					150				155					160	
Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro
				165					170					175	
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
			180					185					190		
Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
		195					200					205			
Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg
	210					215					220				
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val
225				230						235				240	
Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser
			245						250					255	
Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys
			260					265					270		
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp
		275					280						285		
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe
	290					295					300				
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu
305				310					315					320	
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe
				325					330					335	
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly
			340					345						350	

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 355 360 365
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375

<210> 30
 <211> 152
 <212> PRT
 <213> Artificial sequence
 <400> 30

Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly
 1 5 10 15
 Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe
 20 25 30
 Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala
 35 40 45
 Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp
 50 55 60
 Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Val Pro Thr Asp
 65 70 75 80
 Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala
 85 90 95
 Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr
 100 105 110
 Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu
 115 120 125
 Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly
 130 135 140
 Ser His His His His His His His
 145 150

<210> 31
 <211> 379
 <212> PRT
 <213> Artificial sequence
 <400> 31

Met Trp Pro Leu Val Ala Ala Leu Leu Leu Gly Ser Ala Cys Cys Gly
 1 5 10 15
 Ser Ala Gln Leu Leu Phe Asn Lys Thr Lys Ser Val Glu Phe Thr Phe
 20 25 30
 Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr Asn Met Glu Ala
 35 40 45
 Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe Lys Gly Arg Asp
 50 55 60
 Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr Ala Pro Ala Asn
 65 70 75 80
 Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu Lys Gly Asp Ala
 85 90 95
 Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His Thr Gly Asn Tyr
 100 105 110
 Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu Thr Ile Ile Glu
 115 120 125
 Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Ser Gly Gly Gly Gly
 130 135 140
 Ser Ala Ser Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 145 150 155 160
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro

Pro	Lys	Pro	Lys	165	Asp	Thr	Leu	Met	Ile	170	Ser	Arg	Thr	Pro	Glu	175	Val	Thr
Cys	Val	Val	180	Val	Asp	Val	Ser	His	185	Glu	Asp	Pro	Glu	Val	190	Lys	Phe	Asn
Trp	Tyr	Val	195	Asp	Gly	Val	Glu	Val	200	His	Asn	Ala	Lys	Thr	205	Lys	Pro	Arg
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	220	Val	240	
225	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	235	Glu	Tyr	Lys	Cys	Lys	255	Val	Ser
				245						250								
Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys			
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	265	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp		
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe			
290	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu		
305	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe		
				325						330								
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly			
			340					345										
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr			
		355					360					365						
Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								
	370					375												

<210> 32

<211> 152

<212> PRT

<213> Artificial sequence

<400> 32

Met	Trp	Pro	Leu	Val	Ala	Ala	Leu	Leu	Leu	Gly	Ser	Ala	Cys	Cys	Gly
1				5				10					15		
Ser	Ala	Gln	Leu	Leu	Phe	Asn	Lys	Thr	Lys	Ser	Val	Glu	Phe	Thr	Phe
			20					25					30		
Cys	Asn	Asp	Thr	Val	Val	Ile	Pro	Cys	Phe	Val	Thr	Asn	Met	Glu	Ala
		35					40					45			
Gln	Asn	Thr	Thr	Glu	Val	Tyr	Val	Lys	Trp	Lys	Phe	Lys	Gly	Arg	Asp
	50					55					60				
Ile	Tyr	Thr	Phe	Asp	Gly	Ala	Leu	Asn	Lys	Ser	Thr	Ala	Pro	Ala	Asn
65				70					75					80	
Phe	Ser	Ser	Ala	Lys	Ile	Glu	Val	Ser	Gln	Leu	Leu	Lys	Gly	Asp	Ala
			85					90					95		
Ser	Leu	Lys	Met	Asp	Lys	Ser	Asp	Ala	Val	Ser	His	Thr	Gly	Asn	Tyr
			100					105					110		
Thr	Cys	Glu	Val	Thr	Glu	Leu	Thr	Arg	Glu	Gly	Glu	Thr	Ile	Ile	Glu
		115					120					125			
Leu	Lys	Tyr	Arg	Val	Val	Ser	Trp	Phe	Ser	Pro	Ser	Gly	Gly	Gly	Gly
	130					135					140				
Ser	His	His	His	His	His	His	His								
145					150										

<210> 33

```

<211> 1137
<212> DNA
<213> Artificial sequence
<400> 33
atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcagcta
60
ctatttaata aaacaaaatc tgtagaattc acgttttgta atgacactgt cgtcattcca
120
tgctttgtta ctaatatgga ggcacaaaac actactgaag tatacgtaaa gtggaaatth
180
aaaggaagag atatttacac ctttgatgga gctctaaaca agtccactgt cccactgac
240
tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc tttgaagatg
300
gataagagtg atgctgtctc acacacagga aactacactt gtgaagtaac agaattaacc
360
agagaagggtg aaacgatcat cgagctaaaa tatcgtgttg tttcatggtt ttctccatcc
420
ggaggtggag gttccgctag cgagcccaaa tcttgtgaca aaactcacac atgcccaccg
480
tgcccagcac ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaacccaag
540
gacaccctca tgatctcccg gaccctgag gtcacatgcg tgggtggtgga cgtgagccac
600
gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag
660
acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc
720
ctgcaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc
780
ccagccccc a tcgagaaaac catctccaaa gccaaagggc agccccgaga accacagggtg
840
tacaccctgc ctccatctcg ggatgagctg accaagaacc aggtcagcct gacctgcctg
900
gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag
960
aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatagc
1020
aagctcaccg tggacaagag cagggtggcag caggggaacg tcttctcatg ctccgtgatg
1080
catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa
1137

```

```

<210> 34
<211> 457
<212> DNA
<213> Artificial sequence
<400> 34
atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcagcta
60
ctatttaata aaacaaaatc tgtagaattc acgttttgta atgacactgt cgtcattcca
120
tgctttgtta ctaatatgga ggcacaaaac actactgaag tatacgtaaa gtggaaatth
180
aaaggaagag atatttacac ctttgatgga gctctaaaca agtccactgt cccactgac
240

```

tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc tttgaagatg
 300
 gataagagtg atgctgtctc acacacagga aactacactt gtgaagtaac agaattaacc
 360
 agagaagggtg aaacgatcat cgagctaaaa tatcgtgttg tttcatgggtt ttctccatcc
 420
 ggaggtgggtg gatcccatca ccatcaccac catcatt 457

<210> 35

<211> 1137

<212> DNA

<213> Artificial sequence

<400> 35

atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcaacta
 60
 ctatttaata aaacaaaatc tgtagaattc acgttctgta atgacactgt cgtcattcca
 120
 tgctttgtta ctaatatgga ggcacaaaac actaccgaag tatatgtaaa gtggaaattt
 180
 aaaggaagag atatttacac gtttgatgga gctctaaaca agtccactgc ccccgctaac
 240
 tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc tttgaagatg
 300
 gataagagtg atgctgtctc acacacagga aactacactt gtgaagtaac agaattaacc
 360
 agagaagggtg aaacgatcat tgagctaaaa tatcgtgttg tttcgtgggtt ttctccatcc
 420
 ggaggtggag gttccgctag cgagcccaaa tcttgtgaca aaactcacac atgcccaccg
 480
 tgcccagcac ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaaccaag
 540
 gacaccctca tgatctcccg gacccttgag gtcacatgcg tggtggtgga cgtgagccac
 600
 gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag
 660
 acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc
 720
 ctgcaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc
 780
 ccagccccca tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg
 840
 tacaccctgc ctccatctcg ggatgagctg accaagaacc aggtcagcct gacctgcctg
 900
 gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag
 960
 aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatagc
 1020
 aagctcaccg tggacaagag cagggtggcag caggggaacg tcttctcatg ctccgtgatg
 1080
 catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc gggtaaa
 1137

<210> 36

<211> 457

<212> DNA

<213> Artificial sequence

```

<400> 36
atgtggcccc tggtagcggc gctgttgctg ggctcggcgt gctgcggatc agctcaacta
60
ctatttaata aaacaaaatc tgtagaattc acgttctgta atgacactgt cgtcattcca
120
tgctttgtta ctaatatgga ggcacaaaac actaccgaag tatatgtaaa gtggaaattt
180
aaaggaagag atatttacac gtttgatgga gctctaaaca agtccactgc ccccgctaac
240
tttagtagtg caaaaattga agtctcacia ttactaaaag gagatgcctc tttgaagatg
300
gataagagtg atgctgtctc acacacagga aactacactt gtgaagtaac agaattaacc
360
agagaaggtg aaacgatcat tgagctaaaa tatcgtgttg tttcgtggtt ttctccatcc
420
ggaggtggtg gatcccatca ccatcaccac catcatt
457

```

```

<210> 37
<211> 444
<212> PRT
<213> Artificial sequence
<400> 37

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15
Ser Val Gln Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn His
20          25          30
Val Ile His Trp Leu Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35          40          45
Gly Tyr Ile Tyr Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50          55          60
Lys Asp Arg Val Thr Met Thr Ser Asp Thr Ser Ile Ser Thr Ala Tyr
65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Arg Gly Gly Tyr Tyr Thr Tyr Asp Asp Trp Gly Gln Ala Thr Leu
100          105          110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115          120          125
Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
130          135          140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145          150          155          160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165          170          175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180          185          190
Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn
195          200          205
Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
210          215          220
Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe
225          230          235          240
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245          250          255
Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
260          265          270

```

Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro
		275					280					285			
Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr
	290					295					300				
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val
305			310					315					320		
Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala
			325					330					335		
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln
			340					345					350		
Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
		355					360					365			
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro
	370					375					380				
Glu	Asn	Asn	Tyr	Lys	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	
385				390				395						400	
Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu
			405					410						415	
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His
			420				425						430		
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys				
		435					440								

<210> 38

<211> 1332

<212> DNA

<213> Artificial sequence

<400> 38

cagggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgcaggtt
60
tcctgtaagg catctggata caccttcacc aaccatgtta ttcactggct gcgacaggcc
120
cctggacaag ggcttgagtg gatgggatat atttatcctt acaatgatgg tactaagtat
180
aatgagaagt tcaaggacag agtcacgatg acctcagaca cgtccatcag cacagcctac
240
atggagttga gcagtctcag atctgacgac acggccgtct attattgtgc tagagggggg
300
tactatactt acgacgactg gggccaagca accctgggtca ccgtctcgag cgctagcacc
360
aaggggcccat ccgtcttccc cctggcgccc tgctccagga gcacctccga gagcacagcc
420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca
480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcttc aggactctac
540
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcacgaagac ctacacctgc
600
aacgtagatc acaagcccag caacaccaag gtggacaaga gagttgagtc caaatatggt
660
cccccatgcc caccatgccc agcacctgag ttcctggggg gaccatcagt cttcctgttc
720
ccccaaaac ccaaggacac tctcatgac tcccggaccc ctgaggtcac gtgcgtgggtg
780
gtggacgtga gccaggaaga ccccgaggtc cagttcaact ggtacgtgga tggcgtggag
840

gtgcataatg ccaagacaaa gccgcgggag gagcagttca acagcacgta ccgtgtgggc
 900
 agcgtcctca ccgtcctgca ccaggactgg ctgaacggca aggagtacaa gtgcaaggtc
 960
 tccaacaaag gcctcccgtc ctccatcgag aaaaccatct ccaaagccaa agggcagccc
 1020
 cgagagccac aggtgtacac cctgccccca tcccaggagg agatgaccaa gaaccaggtc
 1080
 agcctgacct gcctgggtcaa aggctttctac cccagcgcaca tcgccgtgga gtgggagagc
 1140
 aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc
 1200
 ttcttcctct acagcaggct aaccgtggac aagagcaggt ggcaggaggg gaatgtcttc
 1260
 tcatgctccg tgatgcatga ggctctgcac aaccactaca cacagaagag cctctccctg
 1320
 tctctgggta aa 1332

<210> 39
 <211> 219
 <212> PRT
 <213> Artificial sequence
 <400> 39

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Gly	Lys	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Arg	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	Asp	Asp	Val	Gly	Ile	Tyr	Tyr	Cys	Ser	Gln	Ser
				85					90					95	
Thr	His	Val	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		
Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
		115					120					125			
Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
	130					135					140				
Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
145					150				155					160	
Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
			165						170					175	
Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
			180					185					190		
Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
		195					200					205			
Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys					
	210					215									

<210> 40
 <211> 657
 <212> PRT

<213> Artificial sequence

<400> 40

Gly Ala Thr Ala Thr Thr Gly Thr Gly Ala Thr Gly Ala Cys Thr Cys
 1 5 10 15
 Ala Gly Thr Cys Thr Cys Cys Ala Cys Thr Cys Thr Cys Cys Cys Thr
 20 25 30
 Gly Cys Cys Cys Gly Thr Cys Ala Cys Cys Cys Cys Thr Gly Gly Ala
 35 40 45
 Gly Ala Gly Cys Cys Gly Gly Cys Cys Thr Cys Cys Ala Thr Cys Thr
 50 55 60
 Cys Cys Thr Gly Cys Ala Gly Ala Thr Cys Ala Ala Gly Thr Cys Ala
 65 70 75 80
 Gly Ala Gly Cys Cys Thr Thr Gly Thr Gly Cys Ala Cys Ala Gly Thr
 85 90 95
 Ala Ala Thr Gly Gly Ala Ala Ala Gly Ala Cys Cys Thr Ala Thr Thr
 100 105 110
 Thr Ala Cys Ala Thr Thr Gly Gly Thr Ala Thr Cys Thr Gly Cys Ala
 115 120 125
 Gly Ala Ala Gly Cys Cys Ala Gly Gly Cys Cys Ala Gly Thr Cys Thr
 130 135 140
 Cys Cys Ala Ala Gly Ala Cys Thr Cys Cys Thr Gly Ala Thr Cys Thr
 145 150 155 160
 Ala Cys Ala Ala Ala Gly Thr Thr Thr Cys Cys Ala Ala Cys Cys Gly
 165 170 175
 Ala Thr Thr Thr Thr Cys Thr Gly Gly Thr Gly Thr Cys Cys Cys Ala
 180 185 190
 Gly Ala Cys Ala Gly Ala Thr Thr Cys Ala Gly Cys Gly Gly Cys Ala
 195 200 205
 Gly Thr Gly Gly Gly Thr Cys Ala Gly Gly Cys Ala Cys Thr Gly Ala
 210 215 220
 Thr Thr Thr Cys Ala Cys Ala Cys Thr Gly Ala Ala Ala Thr Cys
 225 230 235 240
 Ala Gly Cys Ala Gly Gly Gly Thr Gly Gly Ala Gly Gly Cys Thr Gly
 245 250 255
 Ala Thr Gly Ala Thr Gly Thr Thr Gly Gly Ala Ala Thr Thr Thr Ala
 260 265 270
 Thr Thr Ala Cys Thr Gly Cys Thr Cys Thr Cys Ala Ala Ala Gly Thr
 275 280 285
 Ala Cys Ala Cys Ala Thr Gly Thr Thr Cys Cys Gly Thr Ala Cys Ala
 290 295 300
 Cys Gly Thr Thr Thr Gly Gly Cys Cys Ala Gly Gly Gly Ala Cys
 305 310 315 320
 Cys Ala Ala Gly Cys Thr Gly Gly Ala Gly Ala Thr Cys Ala Ala Ala
 325 330 335
 Cys Gly Gly Ala Cys Cys Gly Thr Gly Gly Cys Gly Gly Cys Gly Cys
 340 345 350
 Cys Ala Thr Cys Thr Gly Thr Cys Thr Thr Cys Ala Thr Cys Thr Thr
 355 360 365
 Cys Cys Cys Gly Cys Cys Ala Thr Cys Thr Gly Ala Thr Gly Ala Gly
 370 375 380
 Cys Ala Gly Thr Thr Gly Ala Ala Ala Thr Cys Thr Gly Gly Thr Ala
 385 390 395 400
 Cys Cys Gly Cys Thr Ala Gly Cys Gly Thr Thr Gly Thr Gly Thr Gly
 405 410 415
 Cys Cys Thr Gly Cys Thr Gly Ala Ala Thr Ala Ala Cys Thr Thr Cys
 420 425 430

Thr Ala Thr Cys Cys Cys Ala Gly Ala Gly Ala Gly Gly Cys Cys Ala
435 440 445
Ala Ala Gly Thr Ala Cys Ala Gly Thr Gly Gly Ala Ala Gly Gly Thr
450 455 460
Gly Gly Ala Thr Ala Ala Cys Gly Cys Cys Cys Thr Cys Cys Ala Ala
465 470 475 480
Thr Cys Gly Gly Gly Thr Ala Ala Cys Thr Cys Cys Cys Ala Gly Gly
485 490 495
Ala Gly Ala Gly Thr Gly Thr Cys Ala Cys Ala Gly Ala Gly Cys Ala
500 505 510
Gly Gly Ala Cys Ala Gly Cys Ala Ala Gly Gly Ala Cys Ala Gly Cys
515 520 525
Ala Cys Cys Thr Ala Cys Ala Gly Cys Cys Thr Cys Ala Gly Cys Ala
530 535 540
Gly Cys Ala Cys Cys Cys Thr Gly Ala Cys Gly Cys Thr Gly Ala Gly
545 550 555 560
Cys Ala Ala Ala Gly Cys Ala Gly Ala Cys Thr Ala Cys Gly Ala Gly
565 570 575
Ala Ala Ala Cys Ala Cys Ala Ala Ala Gly Thr Cys Thr Ala Cys Gly
580 585 590
Cys Cys Thr Gly Cys Gly Ala Ala Gly Thr Cys Ala Cys Cys Cys Ala
595 600 605
Thr Cys Ala Gly Gly Gly Cys Cys Thr Gly Ala Gly Cys Thr Cys Gly
610 615 620
Cys Cys Cys Gly Thr Cys Ala Cys Ala Ala Ala Gly Ala Gly Cys Thr
625 630 635 640
Thr Cys Ala Ala Cys Ala Gly Gly Gly Gly Ala Gly Ala Gly Thr Gly
645 650 655
Thr

<210> 41
<211> 21
<212> DNA
<213> Artificial sequence
<400> 41
ctcagggaar tarccyttga c 21

<210> 42
<211> 21
<212> DNA
<213> Artificial sequence
<400> 42
tcactgccat caatcttcca c 21

<210> 43
<211> 18
<212> DNA
<213> Artificial sequence
<400> 43
tgtaaaacga cggccagt 18

<210> 44
<211> 18
<212> DNA
<213> Artificial sequence

```

<400> 44
caggaaacag ctatgacc
18

<210> 45
<211> 5
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(5)
<223> Xaa(3)=Val, Phe, Leu; Xaa(4)=Met, Val, Trp, Leu
<400> 45
Asn Tyr Xaa Xaa His
1 5
<210> 46
<211> 17
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(17)
<223> Xaa(10)=Asn, Lys, Cys; Xaa(12)=Asn, Ser, Ala
<400> 46
Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Xaa Tyr Xaa Glu Lys Phe Lys
1 5 10 15
Gly

<210> 47
<211> 8
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(8)
<223> Xaa(3)=Trp, Lys, Thr, Leu; Xaa(4)=Ser, Thr, Lys; Xaa(5)=Met
, Phe, Gly
<400> 47
Gly Gly Xaa Phe Xaa Xaa Asp Tyr
1 5

<210> 48
<211> 5
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(5)
<223> Xaa(3)=Glu, Gly, Thr
<400> 48
Asp Tyr Xaa Met His
1 5

<210> 49
<211> 17
<212> PRT
<213> Artificial sequence

```

11 Jan 2019

2017284157

```
<220>
<221> MOD_RES
<222> (1)..(17)
<223> Xaa(5)=Glu, Tyr, Lys; Xaa(8)=Gly, Asn, Pro; Xaa(13)=Glu, Asn,
Gly, Ser
<400> 49
Ala Ile Asp Pro Xaa Thr Gly Xaa Thr Ala Tyr Asn Xaa Lys Phe Lys
1          5          10          15
Gly
```

```
<210> 50
<211> 8
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(8)
<223> Xaa(4)=Tyr, Lys, Leu
<400> 50
Gly Thr Pro Xaa Ala Met Asp Tyr
1          5
```

```
<210> 51
<211> 5
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(5)
<223> Xaa(3)=Phe, Asn, Tys; Xaa(4)=Leu, Asn, Gly
<400> 51
Asn Tyr Xaa Xaa His
1          5
```

```
<210> 52
<211> 17
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(17)
<223> Xaa(4)=Pro, Gly, Ser, Cys, Lys; Xaa(14)=Asn, Lys
<400> 52
Tyr Ile Asn Xaa Tyr Asn Asp Gly Thr Asn Tyr Asn Glu Xaa Phe Lys
1          5          10          15
Gly
```

```
<210> 53
<211> 10
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(10)
<223> Xaa(2)=Lys, Trp, Phe; Xaa(7)=Ser, Asp, Tyr
```

```

<400> 53
Ala Xaa Gly Gly Trp Phe Xaa Met Asp Tyr
1 5 10

<210> 54
<211> 16
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(16)
<223> Xaa(10)=Asn, Lys, Gly; Xaa(12)=Lys, Gly, Asn; Xaa(16)=Glu,
His, Cys, Lys
<400> 54
Arg Ser Ser Gln Ser Leu Val His Ser Xaa Gly Xaa Thr Tyr Leu Xaa
1 5 10 15

<210> 55
<211> 7
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(7)
<223> Xaa(1)=Lys, Arg
<400> 55
Xaa Val Ser Asn Arg Phe Ser
1 5

<210> 56
<211> 8
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(8)
<223> Xaa(7)=Tyr, Pro, Phe, Asp
<400> 56
Ser Gln Ser Thr His Val Xaa Thr
1 5

<210> 57
<211> 16
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(16)
<223> Xaa(4)=Tyr, Pro, Phe, Asp; Xaa(11)=Gly, Lys, Asn, Ser
<400> 57
Arg Ser Ser Xaa Ser Leu Val His Ser Asn Xaa Asn Thr Tyr Leu His
1 5 10 15

<210> 58
<211> 7
<212> PRT
<213> Artificial sequence

```

```

<220>
<221> MOD_RES
<222> (1)..(7)
<223> Xaa(5)=Arg, Gly, Ser, Lys
<400> 58
Lys Val Ser Asn Xaa Phe Ser
1 5

<210> 59
<211> 9
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(9)
<223> Xaa(4)=Thr, Lys
<400> 59
Ser Gln Ser Xaa His Val Pro Trp Thr
1 5

<210> 60
<211> 16
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(16)
<223> Xaa(6)=Leu, Arg; Xaa(14)=Tyr, Lys, Leu, Gly
<400> 60
Arg Ser Ser Gln Ser Xaa Val His Ser Asn Gly Lys Thr Xaa Leu Gln
1 5 10 15

<210> 61
<211> 7
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(7)
<223> Xaa(2)=Val, Asn, Lys, Ser
<400> 61
Lys Xaa Ser Asn Arg Phe Ser
1 5

<210> 62
<211> 10
<212> PRT
<213> Artificial sequence
<220>
<221> MOD_RES
<222> (1)..(8)
<223> Xaa(2)=Gln, Cys; Xaa(7)=Tys, Pro, Trp, Asp
<400> 62
Ser Xaa Gln Ser Thr His Val Xaa Tyr Thr
1 5 10

```