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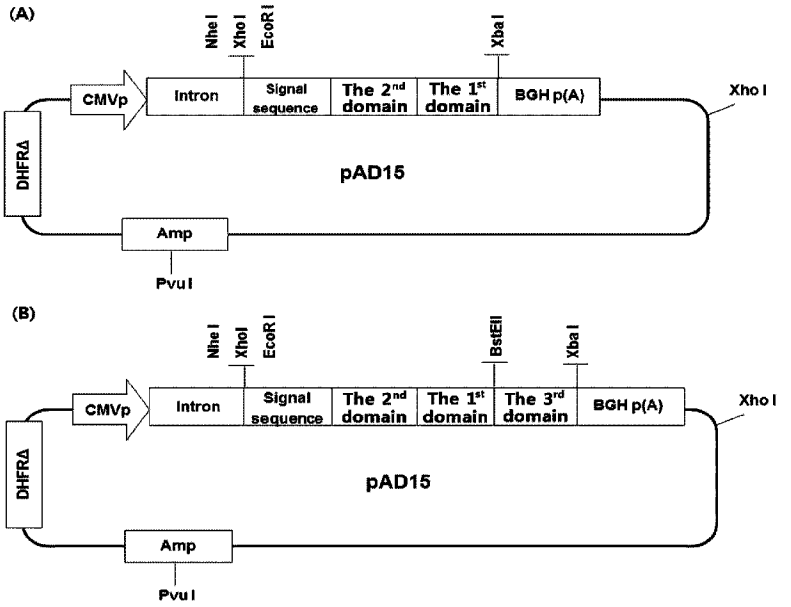
(54) Title: MODIFIED INTERLEUKIN-7 PROTEIN AND USES THEREOF



(57) Abrégé/Abstract:
The present invention provides a modified interleukin-7 and a use thereof. The modified IL-7 or an IL-7 fusion protein of the present invention comprising the same can be obtained in high yield, and biologically active in viral infection and cancer models. Therefore, they can be used for the prevention and treatment of various diseases.

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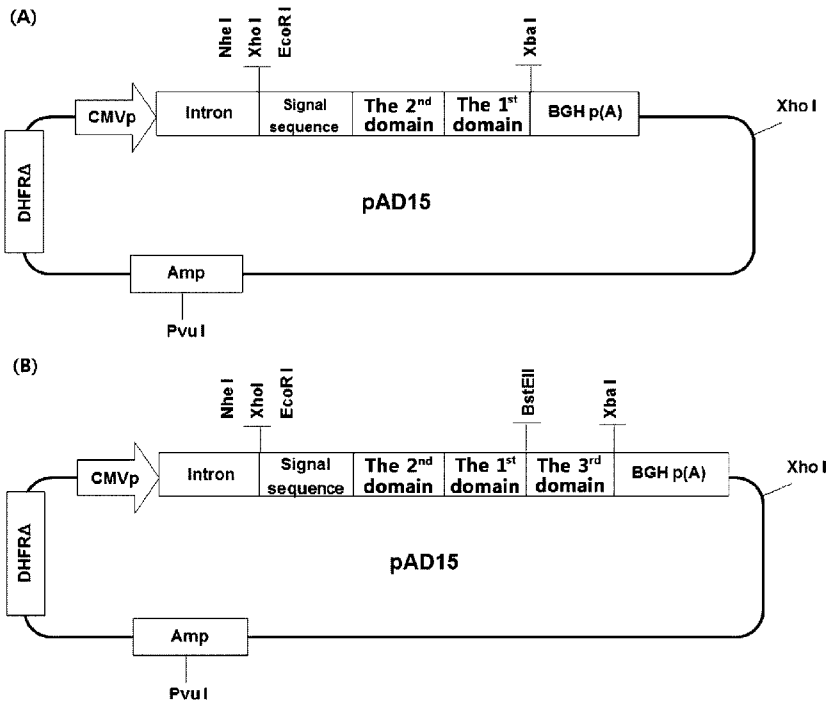

(57) Abstract: The present invention provides a modified interleukin-7 and a use thereof. The modified IL-7 or an IL-7 fusion protein of the present invention comprising the same can be obtained in high yield, and biologically active in viral infection and cancer models. Therefore, they can be used for the prevention and treatment of various diseases.

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Description

Title of Invention: MODIFIED INTERLEUKIN-7 PROTEIN AND USES THEREOF

Technical Field

[1] The present invention relates to modified interleukin-7 protein and uses thereof.

[2]

Background Art

[3] Interleukin-7 or a polypeptide having a similar activity thereto (hereinafter, 'IL-7') is an immunostimulatory cytokine which can promote immune responses mediated by B cells and T cells, and in particular, IL-7 plays an important role in an adaptive immune system. IL-7 is mostly secreted by stromal cells in the bone marrow and thymus, but it is also produced by keratinocytes, dendritic cells, hepatocytes, neurons, and epithelial cells (Heufler C *et al.*, 1993, *J.* Exp. Med. 178 (3): 1109-14; Kroncke R *et al.*, 1996, *Eur.* J. Immunol. 26 (10): 2541- 4: Sawa Y *et al.*, 2009, *Immunity* 30 (3): 447-57; Watanabe M *et al.*, 1995, *J.* Clin. Invest. 95 (6): 2945-53).

[4] Specifically, IL-7 activates immune functions through the survival and differentiation of T cells and B cells, survival of lymphoid cells, stimulation of activity of natural killer (NK) cell, etc., and in particular, IL-7 is important for the development of T cells and B cells. IL-7 binds to hepatocyte growth factor (HGF) and functions as a pre-pro-B cell growth-stimulating factor and a cofactor for V(D)J rearrangement of the T cell receptor beta (TCRβ) (Muegge K, 1993, *Science* 261 (5117): 93-5).

[5] Additionally, IL-7 regulates the development of lymph nodes through lymphoid tissue inducer (LTi) cells and promotes the survival and division of naive T cells or memory T cells. According to the clinical results on viral infection reported recently, IL-7 maintains naive T cells or memory T cells (Amila Patel, *J* Antimicrob Chemother 2010). Furthermore, IL-7 enhances immune response in human by promoting the secretion of IL-2 and interferon-γ.

[6] That is, IL-7 is a cytokine for promoting the survival and proliferation of T cells, B cells, and other immune cells, and it is an excellent candidate material for an immune therapeutic agent which is applicable in various diseases, such as viral infection, cancer, and immune system injury. Recently, several clinical studies on malignancies and human immunodeficiency virus (HIV) infection confirmed the effect of IL-7 on increasing the immunity in human bodies (Fry TJ *et al.*, 2002, *Blood* 99 (11): 3892-904; Muegge K *et al.*, 1993, *Science* 261 (5117): 93-5; Rosenberg SA *et al.*, *J.* Immunother. 29 (3): 313-9). Additionally, IL-7 is also used for the immune recovery after the transplantation of allogenic stem cells (Snyder KM, 2006, *Leuk.* Lymphoma 47 (7): 1222-8)

and the treatment of lymphopenia.

[7] Cancer is life threatening disease. Cancer cells provide an environment that can inhibit immune system so that they can grow without being recognized by immune cells. Cancer patients show an immune deterioration in which T cells are reduced mainly due to anticancer treatment (e.g. chemotherapy, radio-therapy) or showed reduced number at the time of cancer diagnosed. Additionally, although cytotoxic T lymphocytes, effector T cells, and macrophages are gathered inside the cancer tissue, they cannot effectively remove cancer cells. Further, immune cells cannot effectively inhibit the proliferation of cancer cells because T regulatory cells (Treg), myeloid-derived suppression cells (MDSC), *etc.*, which inhibit the function of immune effector cells, are present in cancer tissue.

[8] Under these circumstances, immune therapies are highlighted recently. Immune therapy can be used in combination with chemotherapy or radiation therapy which are currently used for cancer treatment. In particular, the utilization of IL-7 is considered as an alternative for enhancing immune functions by overcoming the lymphopenia in which the number of T cells decrease.

[9] Chronic infection is sustained by inducing exhaustion of T cells that recognize viruses. For example, by being infected with viruses such as HIV, hepatitis B (HBV), hepatitis C (HCV) and simian immunodeficiency virus (SIV), initial immune response is strongly induced, but the functions of virus-specific T cells gradually decrease along with time. In particular, the functions of the virus-specific T cells are reduced by PD-1, LAG-3, TIM-3, IL-10 receptor, TGF-β receptor, *etc.*

[10] However, IL-7 recovers the loss of functions of the virus-specific T cells or inhibits the decrease of their functions by overcoming the immune inhibitory signal system (Pellegrini M, 2009 May; 15 (5): 528-36). Further, IL-7 induces the proliferation of T cells and increases the expression of Bcl-2 thereby promoting the expansion and survival of T cells.

[11] Additionally, IL-7 produces cytokines and helps to retain their functions by inhibiting the expression of SOCS3, which is a mediator for inhibiting cytokine signaling. Further, IL-7 reduces immunopathology due to the production of IL-22 (Som G. Nanjappa, *Blood.* 2011; 117 (19): 5123-5132, Marc Pellegrini, *Cell* 144, 601-613, February 18, 2011).

[12] However, when a recombinant IL-7 is produced for the purpose of medicinal utilization, there are problems in that impurities increase compared to the general recombinant proteins, the amount of IL-7 degradation, and large-scale production cannot be easily achieved. Previously, Cytheris Inc. has been developed a synthetic IL-7, which is a conformer having particular disulfide-bonds (Cys: 1-4; 2-5; 3-6) (U.S. Patent No. 7,585,947). However, since production of synthetic IL-7 requires a com-

plicated denaturation process, the manufacturing process is not easy. Accordingly, there are strong needs for developing a modified IL-7 protein which can be produced in large-scale and by an easy manufacturing process.

[13] In this regard, the modified IL-7, which can be produced in large-scale and by an easy manufacturing process, was manufactured, thereby completing the present invention.

[14]

Disclosure of Invention

Technical Problem

[15] It is an object of the present invention is to provide a modified IL-7.

[16] It is another object of the present invention is to provide a fusion proteins including a modified IL-7.

[17] It is a further object of the present invention is to provide a nucleic acid encoding the modified IL-7 or a fusion proteins including the same, a vector including the nucleic acid and a host cell including the vector, and a method for preparing a modified IL-7 or a fusion proteins including the same.

[18] It is a still further object of the present invention is to provide a pharmaceutical composition comprising the modified IL-7 or a fusion proteins including the same, and uses thereof.

[19]

Solution to Problem

[20] In order to achieve the above objects, the present disclosure relates to a modified IL-7, to which an oligopeptide consisting of 1 to 10 amino acids is linked.

[20a] In one aspect, the present invention provides a modified interleukin-7 having the following structure: A - IL-7, wherein A is linked to the N-terminal of IL-7, wherein A is an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, and a combination thereof, the oligopeptide is not a single methionine, and the IL-7 is an interleukin-7 or a polypeptide having an amino acid sequence identity of 70% or more to SEQ ID NO: 1 and having an interleukin-7 activity.

[21] Additionally, the present disclosure relates to an IL-7 fusion proteins, comprising a first domain comprising a polypeptide having the activity of IL-7 or a similar activity thereof; a

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second domain comprising an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and a third domain which prolongs the half-life of the interleukin-7 fusion proteins.

[21a] In another aspect, the present invention provides an interleukin-7 fusion protein, comprising the following domains (a), (b), and (c): (a) a first domain comprising interleukin-7 or a polypeptide having an amino acid sequence identity of 70% or more to SEQ ID NO: 1 and having an interleukin-7 activity; (b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, and a combination thereof; and (c) a third domain which prolongs the half-life of the interleukin-7 fusion protein, wherein the third domain is selected from the group consisting of an Fc region of an immunoglobulin, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, long unstructured hydrophilic sequences of amino acids (XTEN), an albumin-binding small molecule, and a combination thereof, and wherein the second domain is linked to the N-terminal of the first domain.

[22] Additionally, the present invention provides an isolated nucleic acid molecule encoding the modified IL-7 of the invention or an IL-7 fusion protein of the invention, an expression vector comprising the nucleic acid molecule of the invention, and a host cell comprising the expression vector of the invention.

[23] Additionally, the present disclosure relates to a method of producing or preparing a modified IL-7 or an IL-7 fusion proteins using the nucleic acid, the expression vector, and the host cell.

[23a] In another aspect, the present invention provides a method of preparing a protein, comprising: (a) culturing cells transformed by the expression vector of the invention; and (b) harvesting the modified interleukin-7 or the interleukin-7 fusion protein from the culture or the cells obtained from step (a).

[23b] In another aspect, the present invention provides a method of preparing the modified interleukin-7 of the invention, comprising linking an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, or a combination thereof, wherein the oligopeptide is not a single methionine, to the N-terminal of a polypeptide having the activity of interleukin-7.

[23c] In another aspect, the present invention provides a method of preparing the interleukin-7 fusion protein according to the invention, comprising: linking a second domain comprising an

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amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof, to the N-terminal of a first domain having the activity of interleukin-7; and linking the C-terminal of the first domain to a third domain; wherein the third domain is selected from the group consisting of an Fc region of immunoglobulin, albumin, an albumin-binding polypeptide, PAS, CTP of β subunit of human chorionic gonadotropin, XTEN, an albumin-binding small molecule, and a combination thereof.

[23d] In another aspect, the present invention provides a method of preparing the modified interleukin-7 of the invention, comprising: (a) preparing a linked polynucleotide by linking a polynucleotide encoding an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, or a combination thereof, wherein the oligopeptide is not a single methionine, to the N-terminal of a polynucleotide encoding a polypeptide having the activity of interleukin-7; and (b) harvesting the modified interleukin-7 by expressing the linked polynucleotide.

[23e] In another aspect, the present invention provides a method of preparing the interleukin-7 fusion protein of the invention, comprising: (a) preparing a linked polynucleotide by linking a polynucleotide encoding a polypeptide having the amino acid sequence of a second domain comprising an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof and a polynucleotide encoding a third domain, to the N-terminal and the C-terminal of a polynucleotide encoding a first domain having the activity of interleukin-7, respectively; and (b) harvesting the interleukin-7 fusion protein by expressing the linked polynucleotide, wherein the third domain is selected from the group consisting of an Fc region of immunoglobulin, albumin, an albumin-binding polypeptide, PAS, CTP of β subunit of human chorionic gonadotropin, XTEN, an albumin-binding small molecule, and a combination thereof.

[24] Additionally, the present invention provides a method of preventing or treating a disease using the modified IL-7 or an IL-7 fusion proteins.

[25] In another aspect, the present invention provides a pharmaceutical composition comprising the modified interleukin-7 of the invention or the interleukin-7 fusion protein of the invention, and a pharmaceutically acceptable carrier.

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[25a] In another aspect, the present invention provides the pharmaceutical composition of the invention, for preventing or treating a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

[25b] In another aspect, the present invention provides a use of the modified interleukin-7 of the invention or the interleukin-7 fusion protein of the invention, for prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

[25c] In another aspect, the present invention provides a use of the modified interleukin-7 of the invention or the interleukin-7 fusion protein of the invention, in the manufacture of a medicament for prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

[25d] In another aspect, the present invention provides the modified interleukin-7 of the invention for use in prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

[25e] In another aspect, the present invention provides the interleukin-7 fusion protein of the invention, for use in prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

Advantageous Effects of Invention

[26] The modified IL-7 of the present invention is produced without a denaturation process in high yield. Accordingly, the modified IL-7 of the present invention or a fusion proteins including the same can be applicable in various medicinal fields.

[27]

Brief Description of Drawings

[28] Fig. 1 is a schematic diagram of a gene construct for producing a modified IL-7 of the present invention or a fusion proteins including the same.

[29] Fig. 2 shows the result of the evaluation of daily one cell unit productivity (pg/cell/day, p/c/d) of the IL-7 fusion protein prepared, in which Fig. 2A shows the amount of IL-7-hyFc in culture media and Fig. 2B shows the amount of culturing MGM-IL-7-hyFc in culture media.

[30] Fig. 3 shows the result of comparing the stability between IL-7-hyFc and MGM-IL-7-hyFc including the same according to the various concentrations of sodium chloride.

[31] Fig. 4 shows the result of comparing the native-PAGE between IL-7-hyFc and MGM-IL-7-hyFc according to the various concentrations of sodium chloride.

[32] Fig. 5 shows the result of comparing relative productivity (Fig. 5A) and purity (Fig. 5B) of the prepared IL-7 fusion proteins, respectively.

[33] Fig. 6 shows a graph illustrating the serum drug level according to time after subcutaneous administration of the prepared IL-7 fusion proteins to an SD rat model.

[34] Fig. 7 shows the result of illustrating the production level of anti-drug antibody (ADA) according to each protein administration after subcutaneous administration of the prepared IL-7 fusion proteins to an SD rat model.

[35] Fig. 8 shows a graph illustrating the effect of increasing the number of white blood cells (WBC) according to time after subcutaneous administration of the prepared IL-7 fusion proteins to an SD rat model.

[36] Fig. 9 shows the result of comparing the activity between the prepared IL-7 fusion proteins and the standard material.

[37] Fig. 10 shows a graph illustrating the change in body weight (Fig. 10A) and the change in survival rate (Fig. 10B), in a lethal influenza disease model, according to the administration of the prepared IL-7 fusion proteins.

[38] Fig. 11 shows the images of the morphological observation of anticancer effect, in a cancer cell transplant disease model, according to the administration of the prepared IL-7 fusion proteins.

[39]

Best Mode for Carrying out the Invention

[40] The present invention provides a modified IL-7 having the following structure:

[41] A - IL-7;

[42] wherein A is an oligopeptide consisting of 1 to 10 amino acid residues, and IL-7 is an interleukin 7 or a polypeptide having a similar activity thereto.

[43] As used herein, the term "a polypeptide having the activity of IL-7 or a similar activity thereof" refers to a polypeptide or protein having the same or similar sequence and activity to IL-7. Unless otherwise specified in the present invention, the term can be used as a concept which is interchangeable with the first domain of the IL-7 fusion proteins.

[44] The IL-7 includes a polypeptide consisting of an amino acid sequence represented by SEQ ID NOS: 1 to 6. Additionally, IL-7 may have a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or higher, to the sequences of SEQ ID NOS: 1 to 6.

[45] The IL-7 may include an IL-7 protein or a fragment thereof. In particular, IL-7 may be one derived from humans, rats, mice, monkeys, cows, or sheep.

[46] Specifically, human IL-7 may have an amino acid sequence represented by SEQ ID NO: 1 (Genbank Accession No. P13232); rat IL-7 may have an amino acid sequence represented by SEQ ID NO: 2 (Genbank Accession No. P56478); mouse IL-7 may have an amino acid sequence represented by SEQ ID NO: 3 (Genbank Accession No. P10168); monkey IL-7 may have an amino acid sequence represented by SEQ ID NO: 4 (Genbank Accession No. NP_001279008); cow IL-7 may have an amino acid sequence represented by SEQ ID NO: 5 (Genbank Accession No. P26895), and sheep IL-7 may have an amino acid sequence represented by SEQ ID NO: 6 (Genbank Accession No. Q28540).

[47] Additionally, the IL-7 protein or a fragment thereof may include variously modified proteins or peptides, i.e., variants. The above modification may be performed by a method of a substitution, a deletion, or an addition of at least one protein to the wild type IL-7, without modifying the function of the IL-7. These various proteins or peptides may have a homology of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, to the wild type protein.

[48] Conventionally, a wild type amino acid residue is substituted with alanine, but the substitution may be performed a conservative amino acid substitution, which does not affect or gives a weak effect on the entire protein charge, i.e., polarity or hydrophobicity.

[49] For the conservative amino acid substitution, Table 1 below may be referred to.

[50] [Table 1]

Basic	Arginine (Arg, R)Lysine (Lys, K)Histidine (His, H)
Acidic	Glutamic acid (Glu, E)Aspartic acid (Asp, D)
Uncharged polar	Glutamine (Gln, O)Asparagine (Asn, N)Serine (Ser, S)Threonine (Thr, T)Tyrosine (Tyr, Y)
Non-polar	Phenylalanine (Phe, F)Tryptophan (Trp, W)Cystein (Cys, C)Glycine (Gly, G)Alanine (Ala, A)Valine (Val, V)Proline (Pro, P)Methionine (Met, M)Leucine (Leu, L)NorleucineIsoleucine

[51]

[52] For each amino acid, additional conservative substitution includes "a homolog" of the amino acid. In particular, the "homolog" refers to an amino acid, in which a methylene group (CH_2) is inserted to the side chain of the beta position of the side chain of the amino acid. Examples of the "homolog" may include homophenylalanine, homoarginine, homoserine, etc., but is not limited thereto.

[53] As used herein, the term "IL-7 protein" may be used as a concept to include "IL-7 protein and a fragment thereof" Unless otherwise specified, the terms "protein", "polypeptide", and "peptide" may be used as an interchangeable concept.

[54] In the structure of the modified IL-7, A may be directly linked to the N-terminal of IL-7, or linked through a linker, and unless otherwise specified, the term may be used as a concept which can be interchangeable with the second domain of IL-7 fusion proteins.

[55] In the present invention, A may be linked to the N-terminal of IL-7. The A is characterized in that it includes 1 to 10 amino acids, and the amino acid may be selected from the group consisting of methionine, glycine, and a combination thereof.

[56] Methionine and glycine do not induce immune responses in the human body. The protein therapeutics produced from *E.* coli always include methionine in the N-terminal but no adverse reactions have been reported. Also, glycine is widely used as a GS linker and does not induce immune responses in commercial products as in Dulaglutide (*Cell* Biophys. 1993 Jan-Jun; 22(103):189-224).

[57] In an exemplary embodiment, the A may be an oligopeptide including 1 to 10 amino acids selected from the group consisting methionine (Met, M), glycine (Gly, G), and a

combination thereof, and preferably, an oligopeptide consisting of 1 to 5 amino acids. For example, the A may have N-terminal sequence of any one selected from the group consisting of methionine, glycine, methionine-methionine, glycine-glycine, methionine-glycine, glycine-methionine, methionine-methionine-methionine, methionine-methionine-glycine, methionine-glycine-methionine, glycine-methionine-methionine, methionine-glycine-glycine, glycine-methionine-glycine, glycine-glycine-methionine, and glycine-glycine-glycine. Specifically, the A may be represented by an amino acid sequence selected from the group consisting of methionine, glycine, methionine-methionine, glycine-glycine, methionine-glycine, glycine-methionine, methionine-methionine-methionine, methionine-methionine-glycine, methionine-glycine-methionine, glycine-methionine-methionine, methionine-glycine-glycine, glycine-methionine-glycine, glycine-glycine-methionine, and glycine-glycine-glycine.

[58] Another aspect of the present invention provides an IL-7 fusion protein, comprising: a first domain comprising a polypeptide having the activity of IL-7 or a similar activity thereof; a second domain including an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and a third domain which prolongs the half-life of the interleukin-7 fusion protein.

[59] The third domain may be linked to the N-terminal or the C-terminal of the first domain or the second domain. Additionally, the IL-7 including the first domain and the second domain may be linked to both terminals of the third domain.

[60] The third domain may be a fusion partner for increasing *in* vivo half-life, and preferably, may include any one selected from the group consisting of an Fc region of immunoglobulin or a part thereof, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), C-terminal peptide (CTP) of β subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, and a combination thereof.

[61] When the third domain is an Fc region of immunoglobulin it may be an Fc region of a modified immunoglobulin. In particular, the Fc region of the modified immunoglobulin may be one in which the antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) weakened due to the modification in the binding affinity with the Fc receptor and/or a complement. The modified immunoglobulin may be selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE and a combination thereof. Specifically, the Fc region of the modified immunoglobulin may include a hinge region, a CH2 domain, and a CH3 domain from the N-terminal to the C-terminal. In particular, the hinge region may include the human IgD hinge region; the CH2 domain may include a part of the amino acid residues of the human IgD and a part of the amino acid residues of the human

IgG4 CH2 domain; and the CH3 domain may include a part of the amino acid residues of the human IgG4 CH3 domain.

[62] Additionally, two fusion proteins may form a dimer, for example, when the third domain is an Fc region, the Fc regions may bind to each other and thereby form a dimer.

[63] As used herein, the terms "Fc region", "Fc fragment", or "Fc" refers to a protein which includes the heavy chain constant region 2 (CH2) and the heavy chain constant region 3 (CH3) of immunoglobulin but does not include its variable regions of the heavy chain and the light chain and the light chain constant region (CL1), and it may further include a hinge region of the heavy chain constant region. In the present invention, a hybrid Fc or a hybrid Fc fragment thereof may be called "hFc" or "hyFc."

[64] Additionally, as used herein, the term "an Fc region variant" refers to one which was prepared by substituting a part of the amino acids among the Fc region or by combining the Fc regions of different kinds. The Fc region variant can prevent from being cut off at the hinge region. Specifically, the 144th amino acid and/or 145th amino acid of SEQ ID NO: 9 may be modified. Preferably, the variant may be one, in which the 144th amino acid, K, was substituted with G or S, and one, in which the 145th amino acid, E, was substituted with G or S.

[65]

[66] Additionally, the Fc region or the Fc region variant of the modified immunoglobulin may be represented by the following Formula (I):

[67] [Formula (I)]

[68] N'-(Z1)p-Y-Z2-Z3-Z4-C'.

[69] In the above Formula (I),

[70] N' is the N-terminal of a polypeptide and C' is the C-terminal of a polypeptide;

[71] p is an integer of 0 or 1;

[72] Z1 is an amino acid sequence having 5 to 9 consecutive amino acid residues from the amino acid residue at position 98 toward the N-terminal, among the amino acid residues at positions from 90 to 98 of SEQ ID NO: 7;

[73] Y is an amino acid sequence having 5 to 64 consecutive amino acid residues from the amino acid residue at position 162 toward the N-terminal, among the amino acid residues at positions from 99 to 162 of SEQ ID NO: 7;

[74] Z2 is an amino acid sequence having 4 to 37 consecutive amino acid residues from the amino acid residue at position 163 toward the C-terminal, among the amino acid residues at positions from 163 to 199 of SEQ ID NO: 7;

[75] Z3 is an amino acid sequence having 71 to 106 consecutive amino acid residues from the amino acid residue at position 220 toward the N-terminal, among the amino acid residues at positions from 115 to 220 of SEQ ID NO: 8; and

[76] Z4 is an amino acid sequence having 80 to 107 consecutive amino acid residues from the amino acid residue at position 221 toward the C-terminal, among the amino acid residues at positions from 221 to 327 of SEQ ID NO: 8.

[77]

[78] Additionally, the Fc fragment of the present invention may be in the form of having native sugar chains, increased sugar chains, or decreased sugar chains compared to the native form, or may be in a deglycosylated form. The immunoglobulin Fc sugar chains may be modified by conventional methods such as a chemical method, an enzymatic method, and a genetic engineering method using a microorganism. The removal of sugar chains from an Fc fragment results in a sharp decrease in binding affinity to the C1q part of the first complement component C1, and a decrease or loss of ADCC or CDC, thereby not inducing any unnecessary immune responses *in* vivo. In this regard, an immunoglobulin Fc region in a deglycosylated or aglycosylated form may be more suitable to the object of the present invention as a drug carrier. As used herein, the term "deglycosylation" refers to an Fc region in which sugars are removed enzymatically from an Fc fragment. Additionally, the term "aglycosylation" means that an Fc fragment is produced in an unglycosylated form by a prokaryote, and preferably in *E. coli*.

[79] Additionally, the Fc region of the modified immunoglobulin may include the amino acid sequence of SEQ ID NO: 9 (hyFc), SEQ ID NO: 10 (hyFcM1), SEQ ID NO: 11 (hyFcM2), SEQ ID NO: 12 (hyFcM3), or SEQ ID NO: 13 (hyFcM4). Additionally, the Fc region of the modified immunoglobulin may include the amino acid sequence of SEQ ID NO: 14 (a non-lytic mouse Fc).

[80] According to the present invention, the Fc region of the modified immunoglobulin may be one described in U.S. Patent No. 7,867,491, and the production of the Fc region of the modified immunoglobulin may be performed referring to the disclosure in U.S. Patent No. 7,867,491.

[81] The second domain may be directly linked to the N-terminal of the first domain or linked by a linker. Specifically, the result may be in the form of the second domain-the first domain or the second domain-linker-the first domain.

[82] The third domain may be directly linked to the first domain or the second domain or linked by a linker. Specifically, the result may be in the form of the second domain-the first domain-the third domain, the third domain-the second domain-the first domain, the second domain-the first domain-linker-the third domain, the third domain-linker-the second domain-the first domain, the second domain-linker-the first domain-linker-the third domain, or the third domain-linker-the second domain-the first domain.

[83] When the linker is a peptide linker, the connection may occur in any linking region. They may be coupled using a crosslinking agent known in the art. Examples of the

crosslinking agent may include N-hydroxysuccinimide esters such as 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, and 4-azidosalicylic acid; imidoesters including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane, but is not limited thereto.

[84] Additionally, the linker may be an albumin linker or a peptide linker. The peptide linker may be a peptide of 10 to 20 amino acid residues consisting of Gly and Ser residues.

[85] When the linker is formed by one selected from the group consisting of a chemical bond, the chemical bond may be a disulfide bond, a diamine bond, a sulfide-amine bond, a carboxy-amine bond, an ester bond, and a covalent bond.

[86] In an exemplary embodiment, the modified IL-7 of the present invention may have a structure of A-IL-7 including a polypeptide having the activity of IL-7 or a similar activity thereof and an oligopeptide consisting of 1 to 10 amino acids.

[87] In a specific embodiment, the modified IL-7 may have an amino acid sequence consisting of SEQ ID NOS: 15 to 20. Additionally, the modified IL-7 may have a sequence having a homology of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, to the amino acid sequences consisting of SEQ ID NOS: 15 to 20.

[88] In another exemplary embodiment, the modified IL-7 or an IL-7 fusion protein of the present invention, which comprising a first domain including a polypeptide having the activity of IL-7 or a similar activity thereof; a second domain comprising an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof; and a third domain, which is an Fc region of modified immunoglobulin, coupled to the C-terminal of the first domain.

[89] The IL-7 fusion protein may have an amino acid sequence consisting of SEQ ID NOS: 21 to 25. Additionally, the IL-7 fusion protein may have a sequence having a homology of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, to the amino acid sequences consisting of SEQ ID NOS: 21 to 25.

[90]

[91] Another aspect of the present invention provides an isolated nucleic acid molecule encoding the modified IL-7 or an IL-7 fusion protein.

[92] The nucleic acid molecule may be one encoding the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 15 to 25. The nucleic acid molecule may include a polynucleotide sequence selected from the group consisting of SEQ ID NOS: 29 to 39.

[93] The nucleic acid molecule may further include a signal sequence or a leader sequence.

[94] As used herein, the term "signal sequence" refers to a fragment directing the secretion of a biologically active molecule drug and a fusion protein, and it is cut off after being translated in a host cell. The signal sequence of the present invention is a polynucleotide encoding an amino acid sequence initiating the movement of the protein penetrating the endoplasmic reticulum (ER) membrane. The useful signal sequences in the present invention include an antibody light chain signal sequence, *e.g.*, antibody 14.18 (Gillies *et al.*, *J.* Immunol. Meth 1989. 125:191-202), an antibody heavy chain signal sequence, *e.g.*, MOPC141 an antibody heavy chain signal sequence (Sakano *et al.*, *Nature*, 1980. 286: 676-683), and other signal sequences know in the art (*e.g.*, *see* Watson *et al.*, *Nucleic Acid Research*, 1984. 12:5145-5164).

[95] The characteristics of the signal peptides are well known in the art, and the signal peptides conventionally having 16 to 30 amino acids, but they may include more or less number of amino acid residues. Conventional signal peptides consist of three regions of the basic N-terminal region, a central hydrophobic region, and a more polar C-terminal region.

[96] The central hydrophobic region includes 4 to 12 hydrophobic residues, which immobilize the signal sequence through a membrane lipid bilayer during the translocation of an immature polypeptide. After the initiation, the signal sequence is frequently cut off within the lumen of ER by a cellular enzyme known as a signal peptidase. In particular, the signal sequence may be a secretory signal sequence for tissue plasminogen activation (tPa), signal sequence of herpes simplex virus glycoprotein D (HSV gDs), or a growth hormone. Preferably, the secretory signal sequence used in higher eukaryotic cells including mammals, etc., may be used. Additionally, as the secretory signal sequence, the signal sequence included in the wild type IL-7 may be used or it may be used after substituting with a codon with high expression frequency in a host cell.

[97] Another aspect of the present invention provides an expression vector comprising an isolated nucleic acid molecule encoding the modified IL-7 or an IL-7 fusion protein.

[98] As used herein, the term "vector" is understood as a nucleic acid means which includes a nucleotide sequence that can be introduced into a host cell to be recombined and inserted into the genome of the host cell, or spontaneously replicated as an episome. The vector may include linear nucleic acids, plasmids, phagemids, cosmids, RNA vectors, virus vectors, and analogs thereof. Examples of the virus vectors may include retroviruses, adenoviruses, and adeno-associated viruses, but are not limited thereto.

[99] As used herein, the term "gene expression" or "expression" of a target protein is understood to refer to transcription of a DNA sequence, translation of an mRNA transcript, and secretion of a fusion protein product or a fragment thereof.

[100] As used herein, the term "host cell" refers to a prokaryotic cell and a eukaryotic cell to which a recombinant expression vector can be introduced. As used herein, the terms "transduced", "transformed", and "transfected" refer to the introduction of a nucleic acid (e.g., a vector) into a cell using a technology known in the art.

[101] As used herein, the term "gene expression" or "expression" of a target protein is understood to refer to transcription of a DNA sequence, translation of an mRNA transcript, and secretion of an Fc fusion protein product or an antibody or an antibody fragment thereof.

[102] The useful expression vector may be RcCMV (Invitrogen, Carlsbad) or a variant thereof. The expression vector may include a human cytomegalovirus (CMV) for promoting continuous transcription of a target gene in a mammalian cell and a polyadenylation signal sequence of bovine growth hormone for increasing the stability state of RNA after transcription. In an exemplary embodiment of the present invention, the expression vector is pAD15, which is a modified form of RcCMV.

[103] In another aspect, the present invention provides a host cell including the expression vector. An appropriate host cell can be used for the expression and/or secretion of a target protein, by the transduction or transfection of the DNA sequence of the present invention.

[104] Examples of the appropriate host cell to be used in the present invention may include immortal hybridoma cell, NS/0 myeloma cell, 293 cell, Chinese hamster ovary (CHO) cell, HeLa cell, human amniotic fluid-derived cell (CapT cell) or COS cell.

[105] In still another aspect, the present invention provides a method for producing a protein comprising culturing the transformed cells by the expression vector; and harvesting the modified IL-7 or a fusion protein including IL-7 from the culture or the cells obtained from the culturing process.

[106] The modified IL-7 or a fusion protein including IL-7 may be purified from the culture medium or cell extract. For example, after obtaining the supernatant of the culture medium, in which a recombinant protein was secreted, the supernatant may be concentrated a protein concentration filter available in the commercial market, e.g., an Amicon or Millipore Pellicon ultrafiltration unit. Then, the concentrate may be purified by a method known in the art. For example, the purification may be performed using a matrix coupled to protein A.

[107] In still another aspect, the present invention provides a method for preparing a modified IL-7, including linking an oligopeptide including an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof, to the N-terminal of a polypeptide having the activity of IL-7 or a similar activity thereof.

[108] The above preparation method may further include a step of linking a polypeptide

consisting of a heterogeneous sequence, and the IL-7 fusion protein can be prepared by the same. In particular, the polypeptide consisting of a heterogeneous sequence may be any one selected from the group consisting of an Fc region of immunoglobulin or a part thereof, albumin, an albumin-binding polypeptide, PAS, CTP of the β subunit of human chorionic gonadotropin, PEG, XTEN, HES, an albumin-binding small molecule, and a combination thereof.

[109] In still another aspect, the present invention provides a method for preparing a modified IL-7, comprising: linking a polynucleotide encoding an oligopeptide including an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof, to the N-terminal of a polynucleotide encoding a polypeptide including an amino acid sequence of a first domain having the activity of IL-7 or a similar activity thereof, thereby preparing a linked polynucleotide; and expressing the linked polynucleotide to harvest a modified IL-7 protein.

[110] The above preparation method may further include a step of linking a polynucleotide encoding a polypeptide consisting of a heterogeneous sequence, and the IL-7 fusion protein can be prepared by the same. In particular, the polypeptide consisting of a heterogeneous sequence may be any one selected from the group consisting of an Fc region of immunoglobulin or a part thereof, albumin, an albumin-binding polypeptide, PAS, a CTP of the β subunit of human chorionic gonadotropin, PEG, XTEN, HES, an albumin-binding small molecule, and a combination thereof.

[111] In still another aspect, the present invention provides a pharmaceutical composition for preventing or treating a disease containing the modified IL-7 or an IL-7 fusion protein.

[112] The modified IL-7 or an IL-7 fusion protein of the present invention may be administered for promoting the expansion or survival of naive or pre-existing T-cells or transplanted T-cells, or proliferating the *in* vitro isolated T-cell aggregates.

[113] The diseases may be a chronic hepatitis, cancer, or an infectious disease. The cancer may be head and neck cancer or uterine cervical cancer, and the chronic hepatitis may be hepatitis B or hepatitis C. Additionally, the infectious disease may be a virus infection, and the virus may be selected from the group consisting of influenza virus, CMV, HSV-1, HSV-2, HIV, HCV, HBV, West Nile fever virus, and Dengue virus. Additionally, the disease may be lymphocytopenia (lymphopenia) or any symptom, disease, and symdrome caused by low numbers of lymphocyte, especially T-cells.

[114] The modified IL-7 or an IL-7 fusion protein of the present invention may further include a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may be any non-toxic material which is suitable for the delivery into patients. The carrier may be distilled water, alcohols, fats, waxes, or inactive solids. Additionally, any pharmaceutically acceptable adjuvants (buffering agents, dispersing agents) may

also be contained therein.

[115] Additionally, the pharmaceutical composition containing the modified IL-7 or an IL-7 fusion protein of the present invention may be administered to subjects by various methods. For example, the composition may be parenterally administered, e.g., subcutaneously, intramuscularly, or intravenously. The composition may be sterilized by a conventional sterile method. The composition may contain a pharmaceutically acceptable auxiliary material and an adjuvant required for the regulation of physiological conditions such as pH adjustment, a toxicity-adjusting agent, and an analog thereof. Specific examples may include sodium acetate, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of the fusion protein to be included in the formulations may vary widely. For example, the concentration of the fusion protein may be less than about 0.5%, and generally or at least about 1% to as much as 15% to 20%, depending on the weight. The concentration may be selected based on the selected particular administration methods, fluid volumes, viscosities, etc.

[116] In still another aspect, the present invention provides a method for preventing or treating a disease by administering a composition containing the modified IL-7 or an IL-7 fusion protein of the present invention, as an active ingredient.

[117] The method includes administering a therapeutically effective amount of the modified IL-7 or an IL-7 fusion protein of the present invention to a subject in need thereof, which has a health state directly related or unrelated to the target disease. The subject may be a mammal, and preferably, humans.

[118] The composition of the present invention may be administered in any routes. The composition of the present invention may be provided to animals by a direct administration (e.g., locally by an administration via injection, transplantation, or local administration into a tissue region) or system (e.g., parenterally or orally) via an appropriate means. When the composition of the present invention is administered parenterally by intravenously, subcutaneously, intraocularly, intraperitoneally, intramuscularly, orally, intrarectally, intraorbitally, intracerebrally, intracranially, intraspinally, intraventricularly, intrathecally, intracistenally, intracapsularly, intranasally, or aerosol administration, the composition preferably contains an aqueous or physiologically applicable suspension of body fluids or a part of the solution thereof. As such, the physiologically acceptable carrier or transporter can be added into the composition and delivered to patients, and this does not cause a negative effect on the electrolyte and/or volume balance of patients. Accordingly, the physiologically acceptable carrier or transporter may be a physiological saline.

[119] Additionally, a DNA construct (or a genomic construct) including a nucleic acid including the modified IL-7 or an IL-7 fusion protein of the present invention may be used as a part of the gene therapy protocol.

[120] In the present invention, for reconstituting or complementing the functions of a desired protein, an expression vector capable of expressing a fusion protein in a particular cell may be administered along with any biologically effective carrier. This may be any formulation or composition that can efficiently deliver a gene encoding a desired protein or an IL-7 fusion protein into a cell *in vivo*.

[121] For the purpose of gene therapy using a nucleic acid encoding the modified IL-7 or an IL-7 fusion protein, a subject gene may be inserted into a virus vector, a recombinant bacteria plasmid, or a recombinant eukaryotic plasmid. The virus vector may include a recombinant retrovirus, an adenovirus, an adeno-associated virus, and herpes simplex virus-1, etc. The administration dose of the nucleic acid encoding the fusion protein of the present invention for gene therapy, for humans, may be in the range of 0.1 mg to 200 mg. In an exemplary embodiment, the preferred dose of the nucleic acid encoding the fusion protein of the present invention, for humans, may be in the range of 0.6 mg to 100 mg. In another exemplary embodiment, the preferred dose of the nucleic acid encoding the fusion protein of the present invention, for humans, may be in the range of 1.2 mg to 50 mg.

[122] The unit dose of the modified IL-7 or an IL-7 fusion protein of the present invention may be in the range of 0.001 mg/kg to 10 mg/kg. In an exemplary embodiment, the unit dose of the modified IL-7 or an IL-7 fusion protein may be in the range of 0.01 mg/kg to 2 mg/kg. In another exemplary embodiment, the unit dose of the protein, for humans, may be in the range of 0.02 mg/kg to 1 mg/kg. The unit dose may vary depending on the subject diseases for treatment and the presence of adverse effects. The administration of the modified IL-7 protein may be performed by periodic bolus injections or external reservoirs (e.g., intravenous bags) or by continuous intravenous, subcutaneous, or intraperitoneal administration from the internal (e.g., biocorrosive implants).

[123] The modified IL-7 or an IL-7 fusion protein of the present invention may be administered in combination with other drug(s) or physiologically active material(s) which have a preventative or treating effect on the disease to be prevented or treated, or may be formulated into a combined preparation in combination with other drug(s), for example, may be administered in combination with an immunostimulant such as a hematopoietic growth factor, a cytokine, an antigens, and an adjuvant. The hematopoietic growth factor may be a stem cell factor (SCF), a G-CSF, a GM-CSF, or an Flt-3 ligand. The cytokine may be γ interferon, IL-2, IL-15, IL-21, IL-12, RANTES, or B7-1.

[124] The method of preventing or treating diseases using the composition containing the modified IL-7 or an IL-7 fusion protein of the present invention may also include to administer in combination with other drug(s) or physiologically active material(s), and

the routes for the combined administration, administration period, and dose may be determined depending on the types of diseases, health state of the patient, purpose of treatment or prevention, and other drug(s) or physiologically active material(s) to be administered in combination.

[125]

Mode for the Invention

[126] Hereinafter, the present invention is explained in detail by Examples. The following Examples are intended to further illustrate the present invention without limiting its scope.

[127]

[128] **Example 1. Preparation of a modified IL-7 protein in which an oligopeptide is coupled to IL-7**

[129] A modified IL-7, in which an oligopeptide is coupled to the N-terminal of IL-7 was prepared. For the IL-7, the sequence of human IL-7 (SEQ ID NO: 1) was used and, as an oligopeptide, methionine (M), glycine (G), MM, GG, MG, GM, MMM, MMG, MGM, GMM, MGG, GMG, GGM, GGG, DDD, or MMMM sequence was used.

[130] As shown in Fig. 1A, various forms of the modified IL-7 having the structure of the 'A'-IL-7 were prepared. In this Example, methionine (M), glycine (G), MM, GG, MG, GM, MMM, MMG, MGM, GMM, MGG, GMG, GGM, GGG, DDD, or MMMM sequence was used as the 2nd domain (oligopeptide, 'A'). For the IL-7 as the 1st domain being fused to the oligopeptide, a nucleic acid sequence of SEQ ID NO: 28 was used. An entire nucleic acid sequence in the form where the IL-7 was fused to the oligopeptide was obtained and then inserted into an expression vector. As a negative control, an IL-7 protein without having the oligopeptide modification was prepared in the same manner.

[131] An expression vector including the A-IL-7 gene was transfected into HEK293 cell. Based on the 300 mL of a suspension culture, a polyplex was prepared using 208.3 ㎍ of DNA and 416.6 ㎍ (μL) of polyethylenimine (PEI)(w/w), and then transfected into the HEK293F cell. Six days after the transfection, the cell culture was obtained and subjected to western blot and thereby the expression rate of the target protein was evaluated. Then, the culture was centrifuged at 8,000 rpm for 30 minutes and the culture debris was removed and filtered using a bottle top filter with a pore size of 0.22 ㎛. As a result, the culture liquid containing the modified IL-7 of M-IL-7, G-IL-7, MM-IL-7, GG-IL-7, MG-IL-7, GM-IL-7, MMM-IL-7, MMG-IL-7, MGM-IL-7, GMM-IL-7, MGG-IL-7, GMG-IL-7, GGM-IL-7, GGG-IL-7, DDD-IL-7, and MMMM-IL-7 was obtained.

[132]

[133] **Example** 2. Preparation of an IL-7 fusion protein in which an Fc region is coupled to the C-terminal of IL-7

[134] An IL-7 fusion protein, *i.e.*, the second domain-the first domain-the third domain, in which a polypeptide consisting of a heterogeneous amino acid sequence was further coupled to the C-terminal of a modified IL-7 was prepared. For the first domain, the sequence of human IL-7 (SEQ ID NO: 1) was used, and as the second domain, M, G, MM, GG, MG, GM, MMM, MMG, MGM, GMM, MGG, GMG, GGM, GGG, DDD, or MMMM sequence was used. For the third domain, the sequence of the Fc region (SEQ ID NO: 9 or 14) was used.

[135] As shown in Fig. 1B, various forms of the IL-7 fusion proteins consisting of the second domain, the first domain and the third domain were prepared. In this Example, as the second domain, methionine (M), glycine (G), MM, GG, MG, GM, MMM, MMG, MGM, GMM, MGG, GMG, GGM, GGG, DDD, or MMMM sequence was used; as the first domain, the human IL-7 was used; and as the third domain, hybrid Fc (hFc, hyFc) or mouse non-lytic Fc was used.

[136] In particular, for the hybrid Fc, the hFc (hybrid Fc) disclosed in U.S. Patent No. 7,867,491 was used. The hFc can be coupled to a physiologically active protein and thereby exhibit an excellent *in* vivo half-life compared to the Fc region of the existing modified immunoglobulin.

[137] A gene expression vector was prepared in the same manner as in Example 1 and transfected, and the cells were cultured to prepare a culture liquid containing various forms of IL-7 fusion proteins. As a result, a culture liquid containing G-IL-7-hyFc, M-IL-7-hyFc, MM-IL-7-hyFc, GG-IL-7-hyFc, MG-IL-7-hyFc, GM-IL-7-hyFc, MMM-IL-7-hyFc, MMG-IL-7-hyFc, MGM-IL-7-hyFc, GMM-IL-7-hyFc, MGG-IL-7-hyFc, GMG-IL-7-hyFc, GGM-IL-7-hyFc, GGG-IL-7-hyFc, DDD-IL-7-hyFc, or MMMM-IL-7-hyFc protein was obtained. Additionally, as a control group, a culture liquid containing an IL-7-hyFc protein consisting of the first domain and the third domain was produced.

[138]

[139] **Example 3. Production and purification of modified IL-7 and modified IL-7 fusion protein**

[140] The amount of production of the modified IL-7 proteins and the modified IL-7 fusion proteins produced in Examples above were compared. For each of the fusion proteins, the amount of the proteins in the culture liquid and the amount of proteins present in the cells were measured.

[141] The concentration of the proteins secreted extracellularly was measured by obtaining the cell culture liquid, and the amount of the proteins in the cell was obtained by cell lysis, and the concentration was measured by ELISA method. For the primary

antibody, human IL-7-specific antibody (Southern Biotech, Cat# 10122-01) was used, and as the secondary antibody, Biotin (BD, Cat# 554494) and Streptavidin-HRP (BD, Cat# 554066) were used.

[142] The result of the change in the productivity according to the presence of coupling of an oligopeptide to the N-terminal is shown in Table 2 below.

[143] [Table 2]

The Second Domain	The First Domain	The Third Domain	Average Concentration A in Culture Liquid (μg/mL)	Intracellular Concentration B (μg/mL)	Total Production (A+B)(μg/mL)	Relative Total Production (%)
(non)	IL-7	(non)	53.2	3.8	57.0	100
DDD	IL-7	(non)	37.3	3.0	40.3	71
MGM	IL-7	(non)	63.0	5.9	68.9	121
MGM	IL-7	hFc	154.8	5.3	160.1	281

[144]

[145] As a result, as shown in Table 2, the amount of production of the MGM-IL-7 was increased, compared to IL-7, to which an oligopeptide was not coupled or the DDD-IL-7, to which an amino acid other than methionine and glycine was coupled.

[146] Additionally, when hyFc was further fused to the C-terminal of the modified IL-7, the protein produced was shown to be present at high concentration and the relative total production showed about a 2.8-fold increase.

[147]

[148] **Example 4. Evaluation of productivity of the prepared modified IL-7 and IL-7 fusion proteins**

[149] Among the gene constructs prepared in Example 2, each of the genes of the IL-7-hyFc and MGM-IL-7-hyFc proteins was inserted into pAD15 vector. Then, the pAD15 vector was transfected into the CHO DG44 cell (Columbia University, USA) in adhesion or suspension culture by electroporation method. In the case of adhesion culture, the medium was replaced with a medium containing 10% dFBS (Gibco, USA, 30067-334), MEM alpha (Gibco, 12561, USA, Cat. No. 12561-049), HT (5-hydroxytrypamine, Gibco, USA, 11067-030), five hours after the electroporation. Forty eight hours after the transfection, the medium was replaced with MEM alpha medium containing 10% dFBS without HT, and HT selection was performed. The clones completed with HT selection were subjected to MTX amplification for the amplification of productivity, and the cells were subcultured 2 or 3 times for the stabilization of the cells.

[150] The unit productivity (pg/cell/day, pcd, p/c/d) of the modified IL-7(A-IL-7) and IL-7 fusion protein (A-IL-7-hyFc) was evaluated during the HT selection and MTX amplification. During the subculture, the culture supernatant was recovered and the number of cells was measured, and the amount of each protein was measured from the supernatant using the human IgG ELISA kit (Bethyl, USA). The unit productivity was calculated according to Equation 1 below and the producing cell line was evaluated (pg/cell/day, pcd):

[151] [Equation 1]

[152] $$\text{Unit Productivity (pg/cell/day, pcd)} = \frac{\text{Culture Productivity (pg/mL} \times \text{Total Culture Volume)}}{\text{Number of Survived Cells (cells/T25)} \times \text{Days of Culture (day)}}$$

[153]

[154] Limiting dilution cloning (LDC) was performed using the clones selected by the evaluation of unit productivity, and as a result, the single cell clones with increased productivity were selected. The selected single cell clones were cultured as a suspension cell line using serum-free medium. The long term stability (LTS) test was evaluated by setting a single subculture as 3 days and subculturing 35 times, and the results are shown in Fig. 2.

[155] As shown in Fig. 2A, the IL-7 fusion protein, in which an oligopeptide was not coupled to the N-terminal, did not show an increase in its productivity according to increase of MTX concentration. In this regard, MTX treatment was performed up to the 4 times for further improvement of the productivity, but it was not effective. However, as shown in Fig. 2B, the IL-7 fusion protein (MGM-IL-7-hyFc), in which an oligopeptide was coupled to the N-terminal, showed a significant increase in productivity in an MTX concentration-dependent manner. After the LDL performance, the productivity of the protein was shown to be about 28 pg/cell/day and about 16 μg/mL.

[156] From the above, it was confirmed that the coupling of an oligopeptide to the IL-7 protein could exhibit an excellent effect on the improvement of the productivity of the prepared recombinant IL-7.

[157]

[158] **Example 5. Confirmation of stability of the prepared modified IL-7 and IL-7 fusion proteins**

[159] The culture liquid samples of the IL-7-hyFc and MGM-IL-7-hyFc proteins acquired from the Examples above were purified, and the purified proteins were subjected to size-exclusion (SE) HPLC, and the protein stability according to the concentration of sodium chloride was confirmed.

[160] First, the IL-7-hyFc and MGM-IL-7-hyFc proteins were diluted in a buffer to a concentration of 1 mg/mL. The diluted proteins were filtrated using a 1 mL syringe and a 0.2 μm filter and added into vials. The vials were inserted into an insert and closed by

the vial caps. The proteins were injected into the SE-HPLC system in an amount of 20 μL, respectively. The SE-HPLC was performed under the following conditions, and the purity was confirmed through the values of the peaks obtained thereof.

[161] <SE-HPLC performing conditions>

[162] Column : TSK-GEL G3000SW x L column (7.8 mm × 300 mm) (Tosoh, Japan)

[163] Column Temperature : 25℃

[164] Mobile Phase: a mixed buffer of 50 mM sodium phosphate and 100 mM, 200 mM, or 300 mM sodium chloride (pH 6.8)

[165] Flow rate: 0.6 mL/min

[166] Analysis Time: 40 min

[167] Analysis Method: isocratic method

[168]

[169] As a result, as shown in Fig. 3, the MGM-IL-7-hyFc protein showed a stable pattern although the concentration of sodium chloride changed, compared to the IL-7-hyFc protein.

[170]

[171] **Example 6. Confirmation of native-PAGE of the prepared modified IL-7 and IL-7 fusion proteins**

[172] The difference in stability between the modified IL-7 fusion protein and the IL-7 fusion protein according to the concentration of sodium chloride confirmed in Example 5 was reconfirmed, as follows.

[173] Specifically, native-PAGE was performed according to the conditions described in Table 3 below using IL-7-hyFc and MGM-IL-7-hyFc prepared under the same conditions as in the Examples above. G-CSF-hyFc was used as a control group.

[174] [Table 3]

Gel	Novex® 8-16% Tris-glycine gel (1.5 mm)
Sample Buffer	Tris-glycine Native Sample Buffer (2x)(Invitrogen, LC2673)
Running Buffer	Tris-glycine Native Running Buffer (10x)(Invitrogen, LC2672)
Running Condition	150 V, 5 hr 35 min (in 5℃ cold room)
Amount of Loading	7 μg/well

[175]

[176] As a result as shown in FIG. 4, there was no aggregate occurred in the MGM-IL-7-hyFc protein, compared to the IL-7-hyFc protein, which is consistent with the result of SE-HPLC.

[177]

[178] **Example 7. Analysis of culture samples of the prepared modified IL-7 and IL-7 fusion protein**

[179] The cell lines with the highest productivity among the cell lines, which can produce IL-7-hyFc or MGM-IL-7-hyFc protein, were selected according to the results of Examples 5 and 6. Then, the subcultured culture liquid was obtained and the SE-HPLC was performed by the same method under the same conditions as in Example 5.

[180] As a result, as shown in FIG. 5A, the relative productivity of the MGM-IL-7-hyFc was 2,091%, which was about a 21-fold increase, compared to the amount of the produced IL-7-hyFc protein, which was set as 100%. Additionally, when the amount of the target protein was compared relative to the total proteins produced by each host cell, while the purity of the IL-7-hyFc was about 11.3%, that of the MGM-IL-7-hyFc protein was about 66.4% thus showing about a 6-fold increase (FIG. 5B).

[181] From the result, it was confirmed that coupling an oligopeptide to the IL-7 protein could exhibit an excellent effect on the improvement of purity and productivity of the prepared recombinant IL-7 fusion protein.

[182]

[183] **Example** 8. Pharmacokinetic profiles of the prepared modified IL-7 and IL-7 fusion protein

[184] The pharmacokinetic profiles (PK) were confirmed by comparing the half-life and the area under the curve (AUC) of the prepared IL-7-hyFc and MGM-IL-7-hyFc recombinant protein.

[185] First, male Sprague Dawley (SD) rats (5 rats/group) were subcutaneously administered with each of the recombinant protein in an amount of 0.2 mg/kg, respectively. Blood samples were collected before the administration and at 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after the administration, and stored at room temperature for 30 minutes to aggregate the blood samples. The aggregated blood samples were centrifuged at 3,000 rpm for 10 minutes and the blood serum of each sample was obtained and stored in a deep-freezer.

[186] The samples were analyzed by a test method designed to specifically detect the intact form of the recombinant protein in which no cleavage occurred. Specifically, it is a method for detecting a target protein using the secondary antibody (Southern Biotech, Cat# 9190-05), to which HRP being coupled to the human immunoglobulin G4 (IgG4) of mouse origin is conjugated, after loading a bio-sample containing the prepared recombinant protein into a plate coated with a capture antibody of mouse origin (R&D, Cat# MAB207), which is coupled to the human IL-7. The samples were quantitated by a 10-fold dilution with 1x PBS containing 10% skim milk to be analyzed in a linear position of a standard curve. The results are illustrated in FIG. 6, in terms of the

protein amount remaining in the blood per each time point and the drug concentration area under the curve.

[187] As a result, the IL-7-hyFc and MGM-IL-7-hyFc recombinant proteins showed similar AUC values (about a 1.2-fold). As such, it was confirmed that the fusion of the oligopeptide, which is the second domain, causes no change in pharmacokinetics of the first domain. Therefore, the MGM-IL-7-hyFc recombinant protein and the IL-7-hyFc recombinant protein could exhibit similar pharmacokinetic profile *in vivo*.

[188]

[189] **Example** 9. Immunogenicity of the prepared modified IL-7 and IL-7 fusion proteins

[190] The anti-drug antibody (ADA) producing ability according to the administration of the IL-7-hyFc and MGM-IL-7-hyFc recombinant proteins prepared above was compared, and thereby the antigenicity of each IL-7 fusion protein was examined.

[191] First, the blood samples obtained in the same manner as described in Example 8 were loaded into the plate, which was coated with the IL-7-hyFc or MGM-IL-7-hyFc recombinant proteins in an amount of 0.2 μg/well. Then, the samples were analyzed using the test method designed to detect the ADA in rats using the rat immunoglobulin antibody (Southern Biotech, Cat# 1031-05), to which HRP was conjugated.

[192] In particular, the samples were analyzed by diluting them until the reaction of the ADA became the same as the reaction of the normal rat sera (Negative Cut Off; NCO), and the reaction of the samples according to the dilution fold was measured by optical density. The results are illustrated in FIG. 7.

[193] As a result, as shown in FIG. 7, the dilution fold required for the IL-7-hyFc and the MGM-IL-7-hyFc to arrive at the negative cut off (NCO) was similar. From the result, it was confirmed that the fusion of the oligopeptide, which is the second domain, does not increase antigenicity.

[194]

[195] **Example** 10. Pharmacodynamic profile of the prepared modified IL-7 and IL-7 fusion proteins

[196] The number of white blood cells (WBC) according to the administration of the MGM-IL-7-hyFc and IL-7-hyFc recombinant proteins prepared above was compared, and the pharmacodynamic profile for each of the protein was confirmed.

[197] First, male SD rats (5 rats/group) were subcutaneously administered with each protein in an amount of 0.2 mg/kg. Then, blood samples were collected from the rats before the administration and on the 1st, the 2nd, and the 3rd week after the administration. To prevent the aggregation of the blood samples, the samples were obtained in EDTA-treated tubes, mixed for 5 minutes and stabilized, and the number of WBC was analyzed by complete blood count (CBC) analysis.

[198] As a result, as shown in FIG. 8, the IL-7-hyFc and MGM-IL-7-hyFc recombinant

proteins increased the number of WBC to the highest level in a similar manner on the 2 nd week after the administration. That is, there is no pharmacodynamics change by the fusion of the oligopeptide, which is the second domain. Accordingly, it was confirmed that the IL-7-hyFc and MGM-IL-7-hyFc recombinant proteins can exhibit similar pharmacodynamics profiles.

[199]

[200] **Example** 11. Comparison of *in vitro* activity of the prepared modified IL-7 and IL-7 fusion proteins

[201] The analysis of bioactivity was performed using 2E8 cells (ATCC, TIB-239), which are murine immature B lymphocytes.

[202] First, the cells were seeded into a 96-well plate (1 x 10^5 cells/50 μL/well), and the MGM-IL-7-hyFc was stepwise diluted at a concentration of 750 pM to 2.93 pM and treated on the wells. The cells were cultured in an incubator (37°C, 5% CO_2) for 70 hours, treated with MTS at a concentration of 20 μL/well, and cultured again in the incubator (37°C, 5% CO_2) for 4 hours. Then, the absorbance was measured at 490 nm. In particular, WHO international standard human IL-7 (NIBSC code: 90/530, 100,000 unit) was used as the control group. The calibration curve according to the concentration of the MGM-IL-7-hyFc treatment was created (4-parameter fit) and the result of analysis is shown in FIG. 9.

[203] As a result, as shown in FIG. 9, based on the international standard 100,000 unit, the logEC_{50}-based activity of the MGM-IL-7-hyFc was shown to be 126,000 unit, and the PLA-based activity was shown to be 371,000 unit. This indicates that, considering the number of molecules of IL-7, the activity of the MGM-IL-7-hyFc is similar to or higher than that of the international standard human IL-7.

[204]

[205] **Example 12. Prevention and treatment effect of the prepared modified IL-7 fusion protein in a model infected with a lethal dose of influenza**

[206] The mouse model with a lethal dose of influenza was prepared by anesthetizing followed by the administration with 3LD_{50} H5N2 virus (A/Aquatic bird/Korea/W81/2005) through nasal cavity. Generally, virus-infected mice begin to lose their body weight from 2~3 days and die from a week and thereafter. The thus-prepared disease model was administered with the IL-7 recombinant proteins prepared in Examples 2 and 4 through nasal cavity, and the IL-7-mFc (mouse Fc) (SEQ ID NO: 27) was used as a control group.

[207] In particular, G-CSF can inhibit the early stage influenza infection and proliferate neutrophils thereby promoting immune response. So, G-CSF-hyFc was used as another experimental group.

[208] Six mice were used per each experimental group, and IL-7-mFc (IL-7-mouse Fc),

MGM-IL-7-hyFc, or G-CSF-hyFc was administered through nasal cavity 14 days before the infection with a lethal dose of influenza. Then, $3LD_{50}$ H5N2 virus was administered and the body weight and survival rate were observed for 20 days.

[209] As a result, as shown in FIG. 10, the body weight began to decrease in all groups (FIG. 10A). However, the gap of body weight reduction was gradually decreased in the group treated with IL-7-mFc and the group treated with MGM-IL-7-hyFc and gradually recovered body weight, and their survival rate was high unlike that of the control group. The group treated with IL-7-mFc survived 100% and the group treated with MGM-IL-7-hyFc showed a 83% survival rate (FIG. 10B).

[210] The reason that the effect of MGM-IL-7-hyFc is observed slightly lower than that of IL-7-mFc appears to be due to the difference between species. That is, in the *in* vivo system of a mouse, the human-derive Fc has lower function than that of a mouse-derived Fc.

[211] Meanwhile, the group treated with PBS or G-CSF-hyFc showed a very low effect and thus the survival rate of the mice infected with a lethal dose of influenza in 10 days was 0%.

[212] In conclusion, the MGM-IL-7-hyFc recombinant proteins showed a strong effect in an influenza model, but G-CSF-hyFc did not, indicating that MGM-IL-7 is very effective in influenza.

[213]

[214] **Example** 13. Treatment effect of the prepared modified IL-7 fusion protein in a model with TC-1 cancer disease

[215] In order to prepare a disease model with endometrial cancer, mice were administered intraperitoneally with 3 mg of Depo-Provera to adjust the period of their menstruation. In 4 days, the mice were administered with nonoxynol-9 (N9, USP, Cat. No. 1467950) into the vagina to stimulate the vagina tissue, and the remaining N9 was removed by washing with PBS. Then, for the transplant of the cancer cells into the uterus, $1x10^5$ TC-1 cell (Dr. Jae-Tae LEE, School of Medicine, Kyungpook National University) were administered, and then 1 day thereafter, 1 μg of MGM-IL-7-hyFc was administered into the uterine cervix.

[216] As a result, 28 days after the administration of a fusion protein, the TC-1 tumor cells were engrafted to the uterine cervix or inside the vagina and grew. That is, as shown in FIG. 11, the cancer cells proliferated and exposed outside of the vaginal orifice. In the case of the control group administered with PBS, 6 out of 10 mice were observed to have the proliferation of the TC-1 cells, and one among them died due to excessive proliferation of the cancer cells.

[217] In contrast, in the experimental group administered with the MGM-IL-7 fusion protein, only two out of 10 mice were observed to have the symptom of the pro-

liferation of the TC-1 cells and no mice were dead. Accordingly, it was confirmed that the modified IL-7 fusion protein is effective for the prevention and treatment of cancer.

Claims

1. A modified interleukin-7 having the following structure:
A - IL-7
wherein A is linked to the N-terminal of IL-7,
wherein A is an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, and a combination thereof,
the oligopeptide is not a single methionine, and
the IL-7 is an interleukin-7 or a polypeptide having an amino acid sequence identity of 70% or more to SEQ ID NO: 1 and having an interleukin-7 activity.

2. The modified interleukin-7 of claim 1, wherein the IL-7 does not comprise a signal peptide.

3. The modified interleukin-7 of claim 1 or 2, wherein the IL-7 comprises amino acid residues 26-177 of SEQ ID NO: 1.

4. The modified interleukin-7 of claim 1, wherein the IL-7 has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 6.

5. The modified interleukin-7 of any one of claims 1 to 4, wherein A is selected from the group consisting of glycine, methionine-methionine, glycine-glycine, methionine-glycine, glycine-methionine, methionine-methionine-methionine, methionine-methionine-glycine, methionine-glycine-methionine, glycine-methionine-methionine, methionine-glycine-glycine, glycine-methionine-glycine, glycine-glycine-methionine, and glycine-glycine-glycine.

6. An interleukin-7 fusion protein, comprising the following domains (a), (b), and (c):
(a) a first domain comprising interleukin-7 or a polypeptide having an amino acid sequence identity of 70% or more to SEQ ID NO: 1 and having an interleukin-7 activity;
(b) a second domain comprising an oligopeptide having 1 to 10 amino acid residues selected from

the group consisting of methionine, glycine, and a combination thereof; and
(c) a third domain which prolongs the half-life of the interleukin-7 fusion protein, wherein the third domain is selected from the group consisting of an Fc region of an immunoglobulin, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, long unstructured hydrophilic sequences of amino acids (XTEN), an albumin-binding small molecule, and a combination thereof, and
wherein the second domain is linked to the N-terminal of the first domain.

7. The interleukin-7 fusion protein of claim 6, wherein the interleukin-7 or the polypeptide does not comprise a signal peptide.

8. The interleukin-7 fusion protein of claim 6 or 7, wherein the interleukin-7 or the polypeptide comprises amino acid residues 26-177 of SEQ ID NO: 1.

9. The interleukin-7 fusion protein of any one of claims 6 to 8, wherein the third domain is linked to the N-terminal or C-terminal of the first domain or the second domain.

10. The interleukin-7 fusion protein of any one of claims 6 to 9, wherein the second domain is selected from the group consisting of methionine, glycine, methionine-methionine, glycine-glycine, methionine-glycine, glycine-methionine, methionine-methionine-methionine, methionine-methionine-glycine, methionine-glycine-methionine, glycine-methionine-methionine, methionine-glycine-glycine, glycine-methionine-glycine, glycine-glycine-methionine, and glycine-glycine-glycine.

11. The interleukin-7 fusion protein of any one of claims 6 to 10, wherein the third domain comprises an Fc region of a modified immunoglobulin.

12. The interleukin-7 fusion protein of claim 11, wherein the modified immunoglobulin is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE and a combination thereof.

13. The interleukin-7 fusion protein of claim 11, wherein the Fc region of the modified immunoglobulin comprises a hinge region, a CH2 domain, and a CH3 domain from the N-terminal to the C-terminal direction,
wherein the hinge region comprises a human IgD hinge region,
the CH2 domain comprises a part of the amino acid residues of CH2 domain of human IgD and human IgG4, and
the CH3 domain comprises a part of the amino acid residues of the human IgG4 CH3 domain.

14. The interleukin-7 fusion protein of claim 11, wherein the Fc region of the modified immunoglobulin is represented by the following Formula (I):
[Formula (I)]
N'-(Z1)p-Y-Z2-Z3-Z4-C'
wherein N' is the N-terminal of a polypeptide and C' is the C-terminal of a polypeptide;
p is an integer of 0 or 1;
Z1 is an amino acid sequence having 5 to 9 consecutive amino acid residues from the amino acid residue at position 98 toward the N-terminal, among the amino acid residues at positions from 90 to 98 of SEQ ID NO: 7;
Y is an amino acid sequence having 5 to 64 consecutive amino acid residues from the amino acid residue at position 162 toward the N-terminal, among the amino acid residues at positions from 99 to 162 of SEQ ID NO: 7;
Z2 is an amino acid sequence having 4 to 37 consecutive amino acid residues from the amino acid residue at position 163 toward the C-terminal, among the amino acid residues at positions from 163 to 199 of SEQ ID NO: 7;
Z3 is an amino acid sequence having 71 to 106 consecutive amino acid residues from the amino acid residue at position 220 toward the N-terminal, among the amino acid residues at positions from 115 to 220 of SEQ ID NO: 8; and
Z4 is an amino acid sequence having 80 to 107 consecutive amino acid residues from the amino acid residue at position 221 toward the C-terminal, among the amino acid residues at positions from 221 to 327 of SEQ ID NO: 8.

15. The interleukin-7 fusion protein of claim 6, wherein the third domain has an amino acid sequence selected from the group consisting of SEQ ID NOS: 9 to 14.

16. An isolated nucleic acid molecule encoding the modified interleukin-7 according to any one of claims 1 to 5 or the interleukin-7 fusion protein according to any one of claims 6 to 15.

17. The nucleic acid molecule of claim 16, wherein the nucleic acid molecule encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 15 to 25.

18. The nucleic acid molecule of claim 16, wherein the nucleic acid molecule comprises a polynucleotide having a base sequence selected from the group consisting of SEQ ID NOS: 29 to 39.

19. An expression vector comprising the nucleic acid molecule according to any one of claims 16 to 18.

20. A host cell comprising the expression vector according to claim 19.

21. A method of preparing a protein, comprising:
(a) culturing cells transformed by the expression vector according to claim 19; and
(b) harvesting the modified interleukin-7 or the interleukin-7 fusion protein from the culture or the cells obtained from step (a).

22. A method of preparing the modified interleukin-7 according to any one of claims 1 to 5, comprising linking an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, or a combination thereof, wherein the oligopeptide is not a single methionine, to the N-terminal of a polypeptide having the activity of interleukin-7.

23. A method of preparing the interleukin-7 fusion protein according to any one of claims 6 to 15, comprising:
linking a second domain comprising an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof, to the N-terminal of a first domain having the activity of interleukin-7; and
linking the C-terminal of the first domain to a third domain;
wherein the third domain is selected from the group consisting of an Fc region of immunoglobulin, albumin, an albumin-binding polypeptide, PAS, CTP of β subunit of human chorionic gonadotropin, XTEN, an albumin-binding small molecule, and a combination thereof.

24. A method of preparing the modified interleukin-7 according to any one of claims 1 to 5, comprising:
(a) preparing a linked polynucleotide by linking a polynucleotide encoding an oligopeptide consisting of 1 to 10 amino acid residues selected from the group consisting of methionine, glycine, or a combination thereof, wherein the oligopeptide is not a single methionine, to the N-terminal of a polynucleotide encoding a polypeptide having the activity of interleukin-7; and
(b) harvesting the modified interleukin-7 by expressing the linked polynucleotide.

25. A method of preparing the interleukin-7 fusion protein according to any one of claims 6 to 15, comprising:
(a) preparing a linked polynucleotide by linking a polynucleotide encoding a polypeptide having the amino acid sequence of a second domain comprising an amino acid sequence having 1 to 10 amino acid residues consisting of methionine, glycine, or a combination thereof and a polynucleotide encoding a third domain, to the N-terminal and the C-terminal of a polynucleotide encoding a first domain having the activity of interleukin-7, respectively; and
(b) harvesting the interleukin-7 fusion protein by expressing the linked polynucleotide,
wherein the third domain is selected from the group consisting of an Fc region of immunoglobulin, albumin, an albumin-binding polypeptide, PAS, CTP of β subunit of human chorionic gonadotropin, XTEN, an albumin-binding small molecule, and a combination thereof.

26. A pharmaceutical composition comprising the modified interleukin-7 according to any one of claims 1 to 5 or the interleukin-7 fusion protein according to any one of claims 6 to 15, and a pharmaceutically acceptable carrier.

27. The pharmaceutical composition of claim 26, for preventing or treating a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

28. The pharmaceutical composition of claim 27, wherein the cancer is head and neck cancer, or uterine cervical cancer.

29. A use of the modified interleukin-7 defined in any one of claims 1 to 5 or the interleukin-7 fusion protein defined in any one of claims 6 to 15, for prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

30. A use of the modified interleukin-7 defined in any one of claims 1 to 5 or the interleukin-7 fusion protein defined in any one of claims 6 to 15, in the manufacture of a medicament for prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

31. The use of claim 29 or 30, wherein the cancer is head and neck cancer, or uterine cervical cancer.

32. The modified interleukin-7 defined in any one of claims 1 to 5 for use in prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

33. The modified interleukin-7 for use of claim 32, wherein the cancer is head and neck cancer, or uterine cervical cancer.

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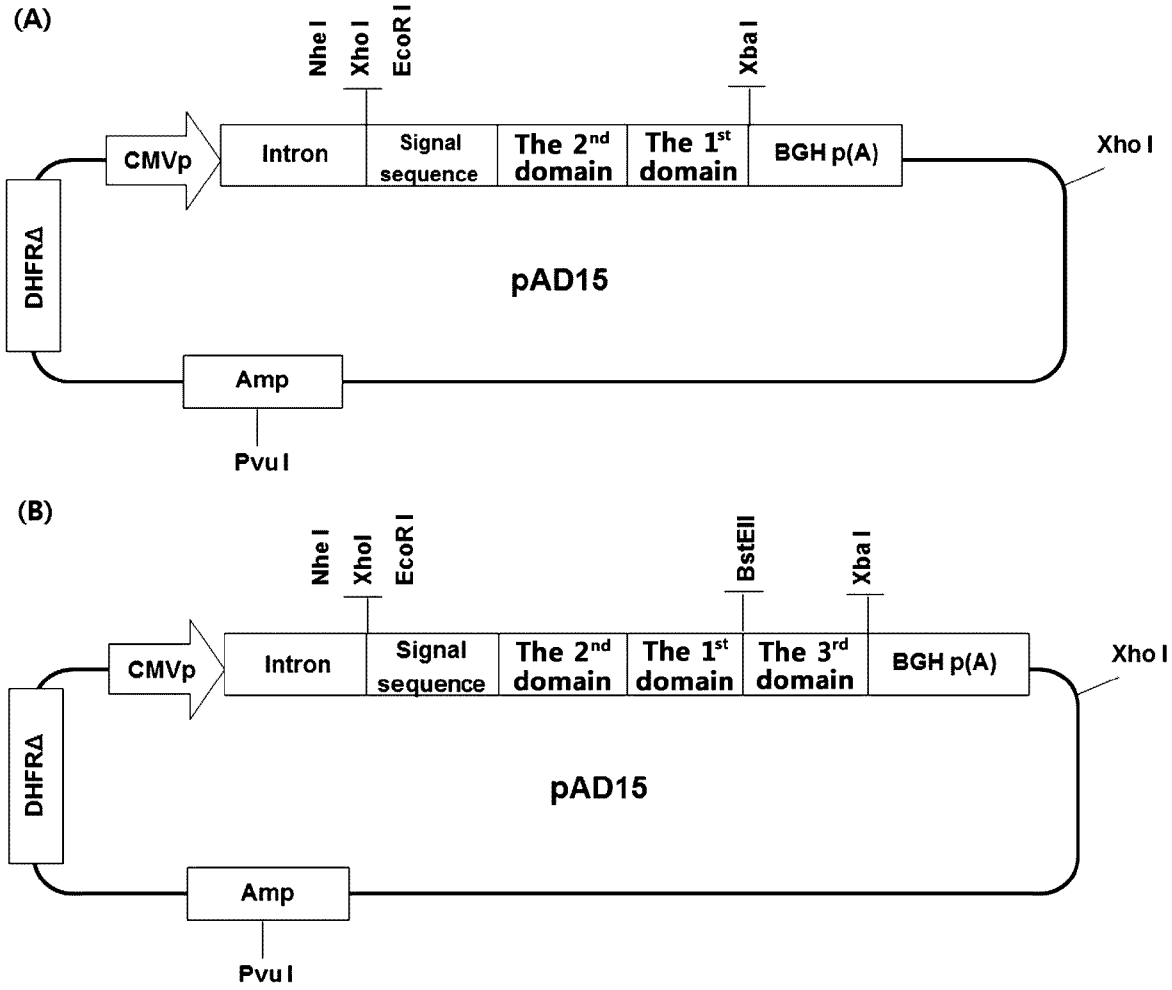
34. The interleukin-7 fusion protein defined in any one of claims 6 to 15, for use in prevention or treatment of a disease selected from the group consisting of cancer, influenza, hepatitis B, hepatitis C, lymphocytopenia (lymphopenia) and virus infection.

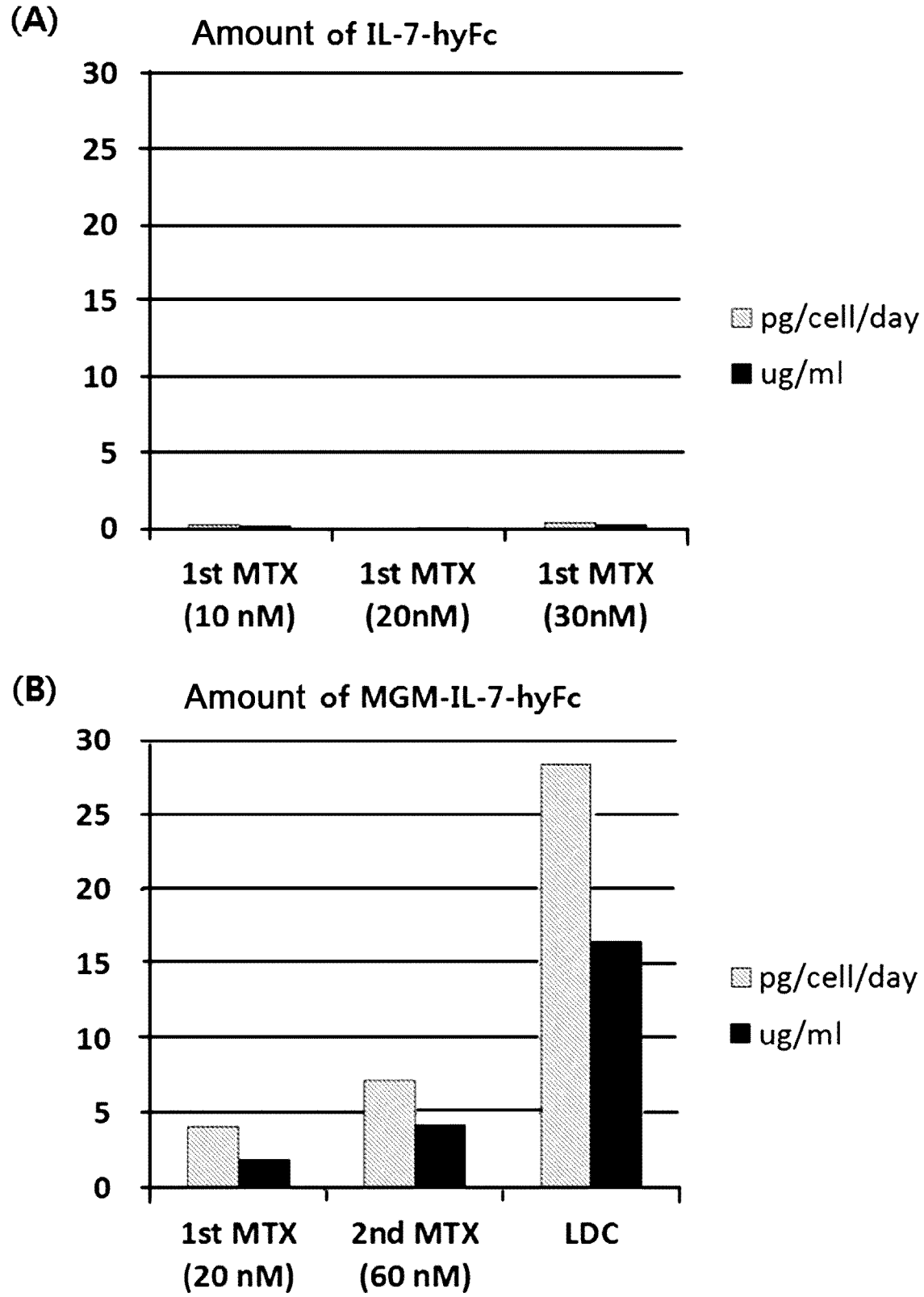
35. The interleukin-7 fusion protein for use of claim 34, wherein the cancer is head and neck cancer, or uterine cervical cancer.

Date Reçue/Date Received 2023-08-28

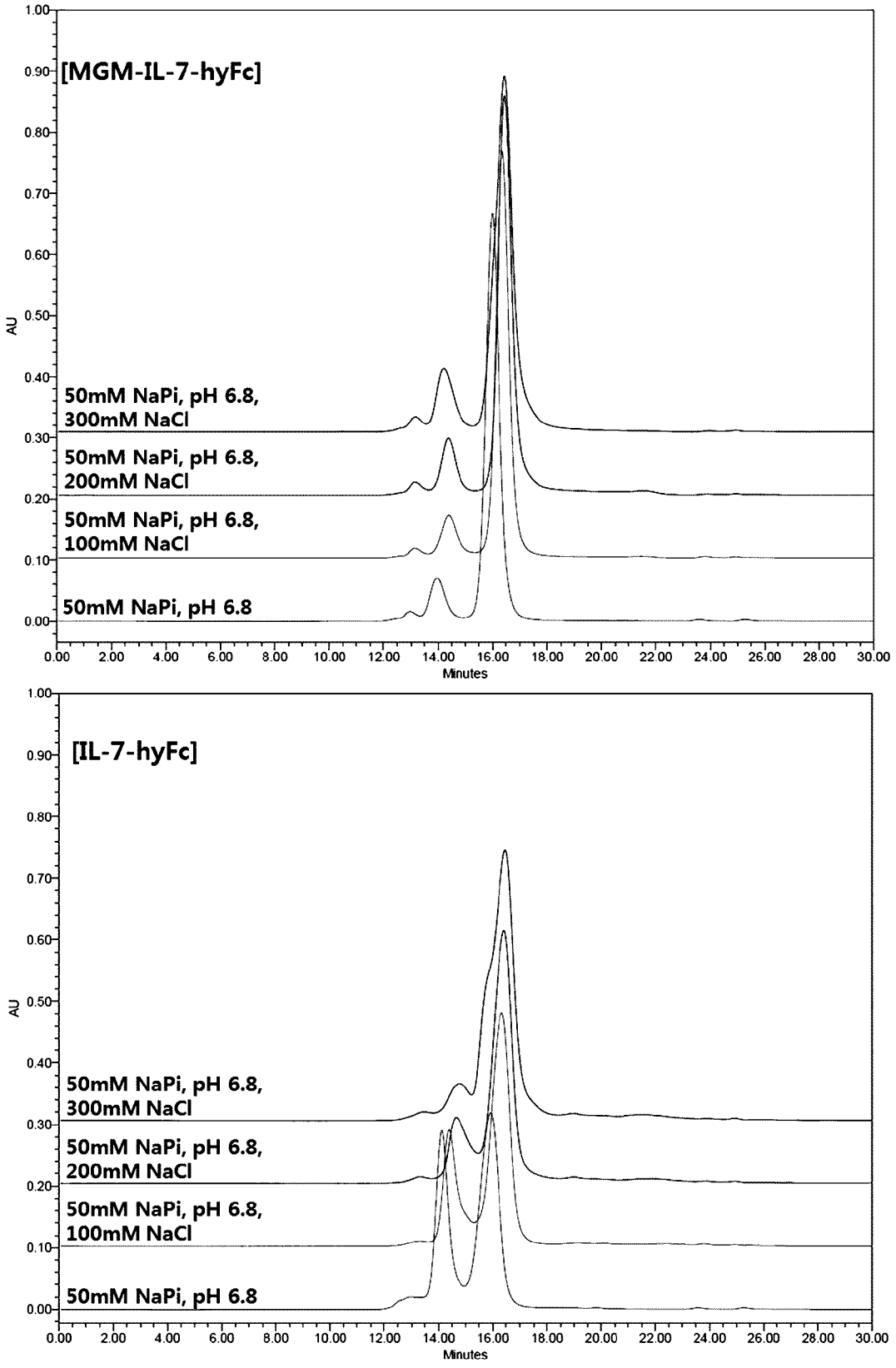
[Fig. 1]


(A)
Nhe I
Xho I
EcoR I
Xba I
CMVp
Intron
Signal sequence
The 2nd domain
The 1st domain
BGH p(A)
Xho I
DHFRΔ
pAD15
Amp
Pvu I
(B)
Nhe I
XhoI
EcoR I
BstEII
Xba I
CMVp
Intron
Signal sequence
The 2nd domain
The 1st domain
The 3rd domain
BGH p(A)
Xho I
DHFRΔ
pAD15
Amp
Pvu I

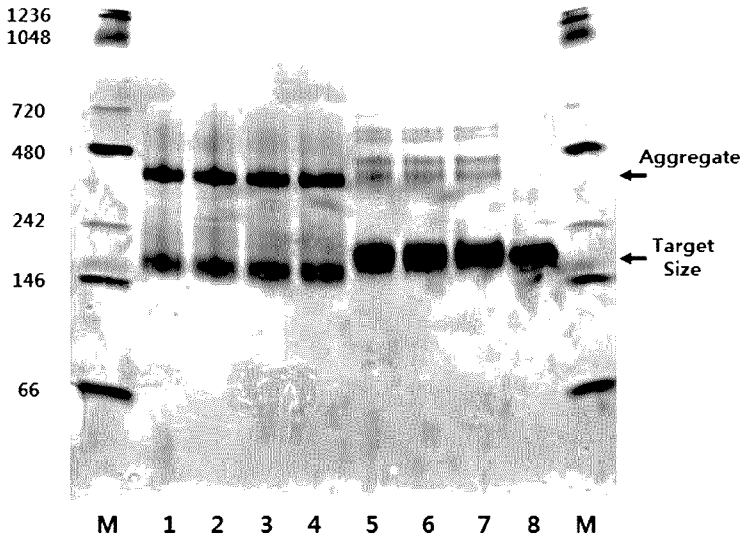
[Fig. 2]


(A)
Amount of IL-7-hyFc
30
25
20
15
10
5
0
1st MTX (10 nM)
1st MTX (20nM)
1st MTX (30nM)
pg/cell/day
ug/ml
(B)
Amount of MGM-IL-7-hyFc
30
25
20
15
10
5
0
1st MTX (20 nM)
2nd MTX (60 nM)
LDC
pg/cell/day
ug/ml

[Fig. 3]

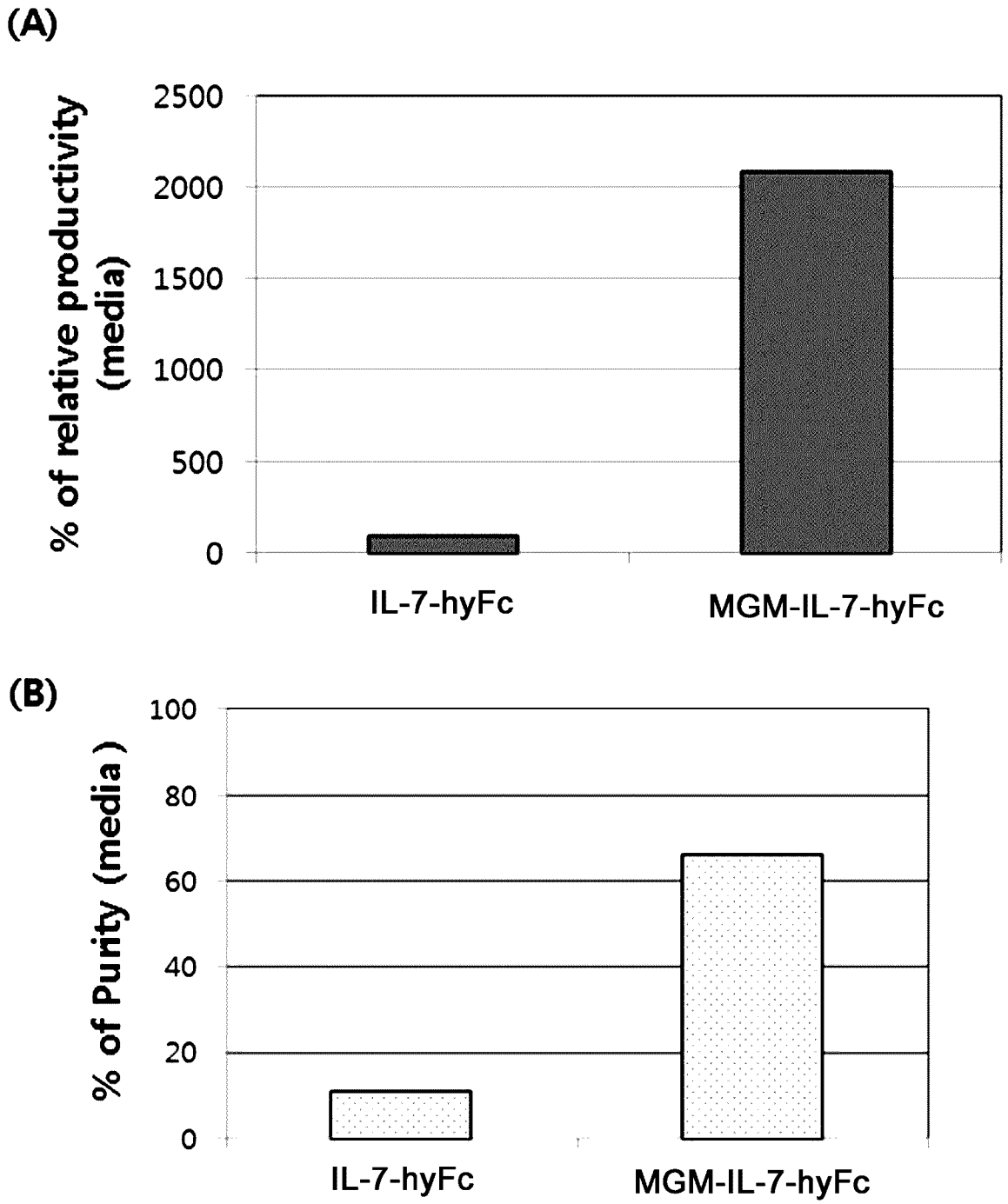

[MGM-IL-7-hyFc]
50mM NaPi, pH 6.8, 300mM NaCl
50mM NaPi, pH 6.8, 200mM NaCl
50mM NaPi, pH 6.8, 100mM NaCl
50mM NaPi, pH 6.8
AU
Minutes
[IL-7-hyFc]
50mM NaPi, pH 6.8, 300mM NaCl
50mM NaPi, pH 6.8, 200mM NaCl
50mM NaPi, pH 6.8, 100mM NaCl
50mM NaPi, pH 6.8
AU
Minutes

[Fig. 4]

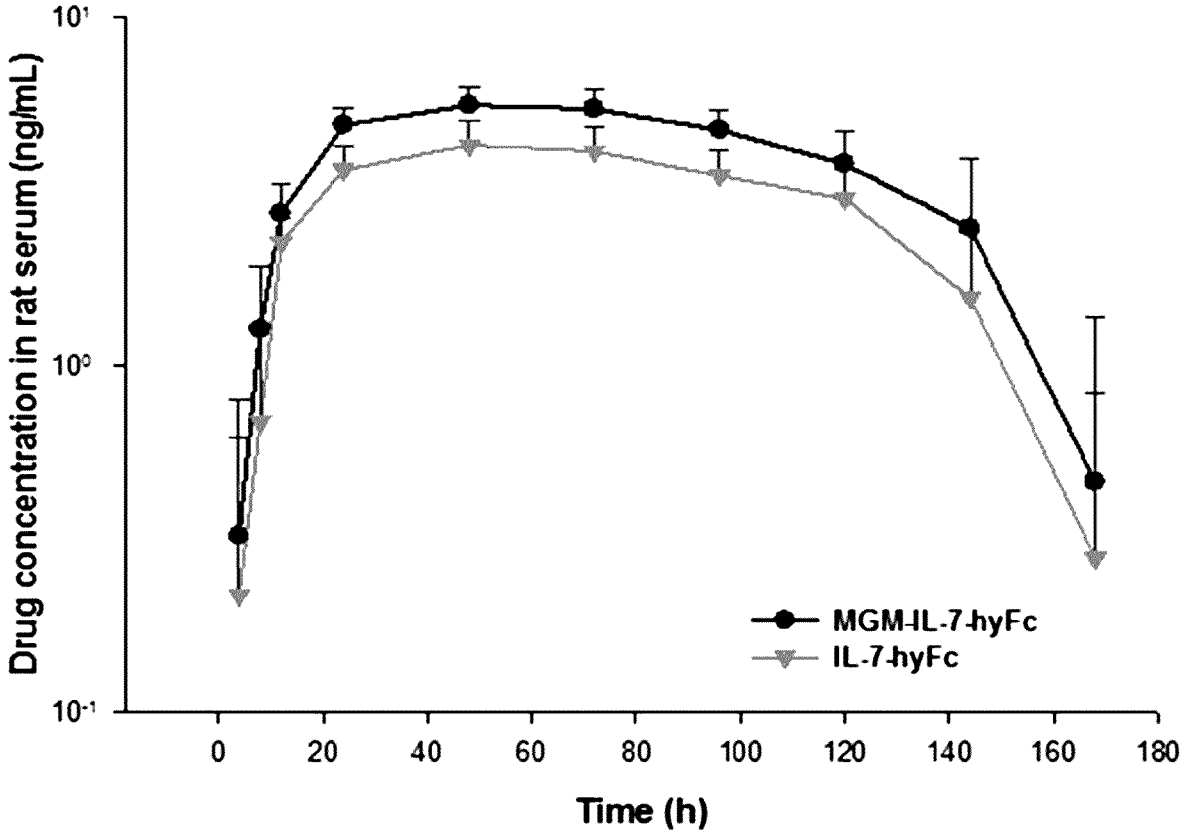


Lane	Sample (7ug/well)	NaCl Conc.
M	Marker	
1	IL-7-hyFc	N/A
2		100mM
3		200mM
4		300mM
5	MGM-IL-7-hyFc	N/A
6		100mM
7		300mM
8	G-CSF-hyFc (control)	N/A

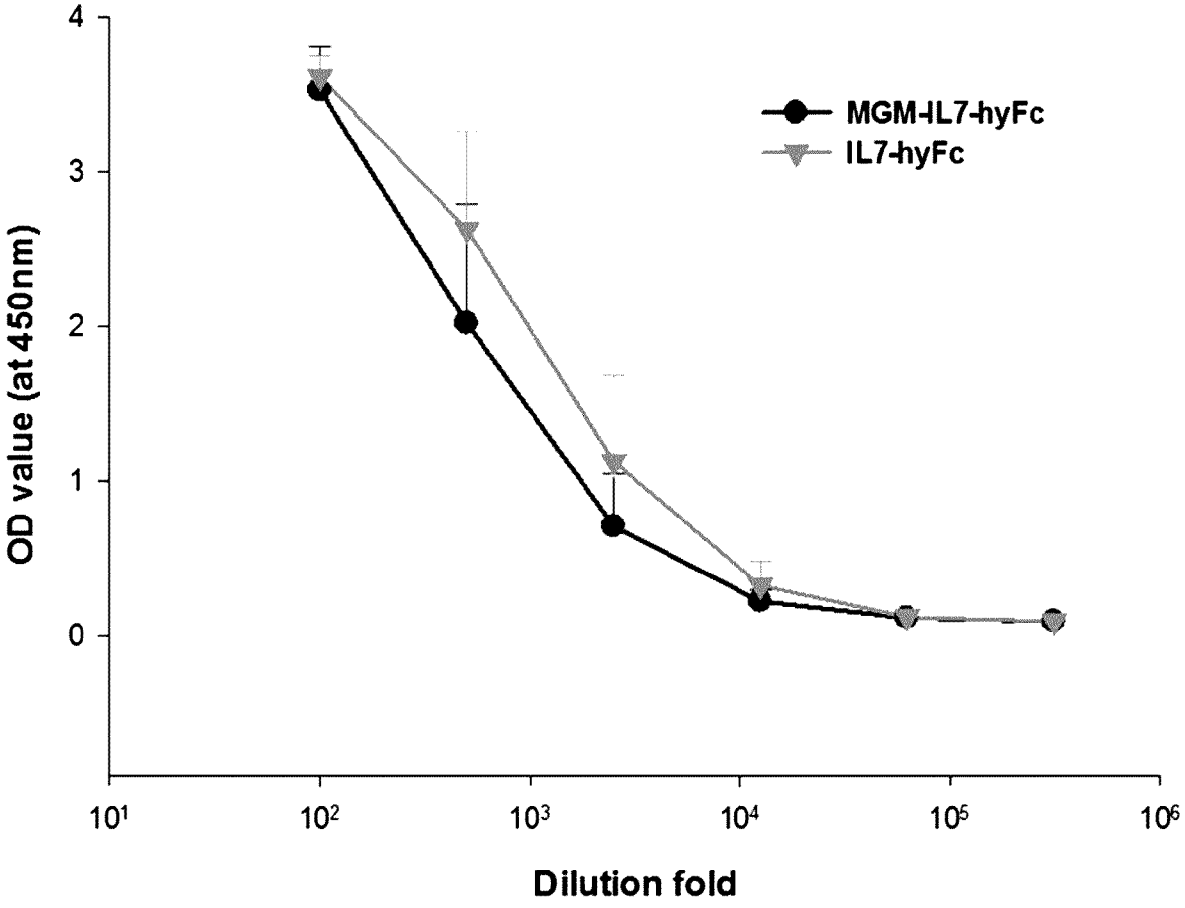
[Fig. 5]


(A)
% of relative productivity (media)
2500
2000
1500
1000
500
0
IL-7-hyFc
MGM-IL-7-hyFc
(B)
% of Purity (media)
100
80
60
40
20
0
IL-7-hyFc
MGM-IL-7-hyFc

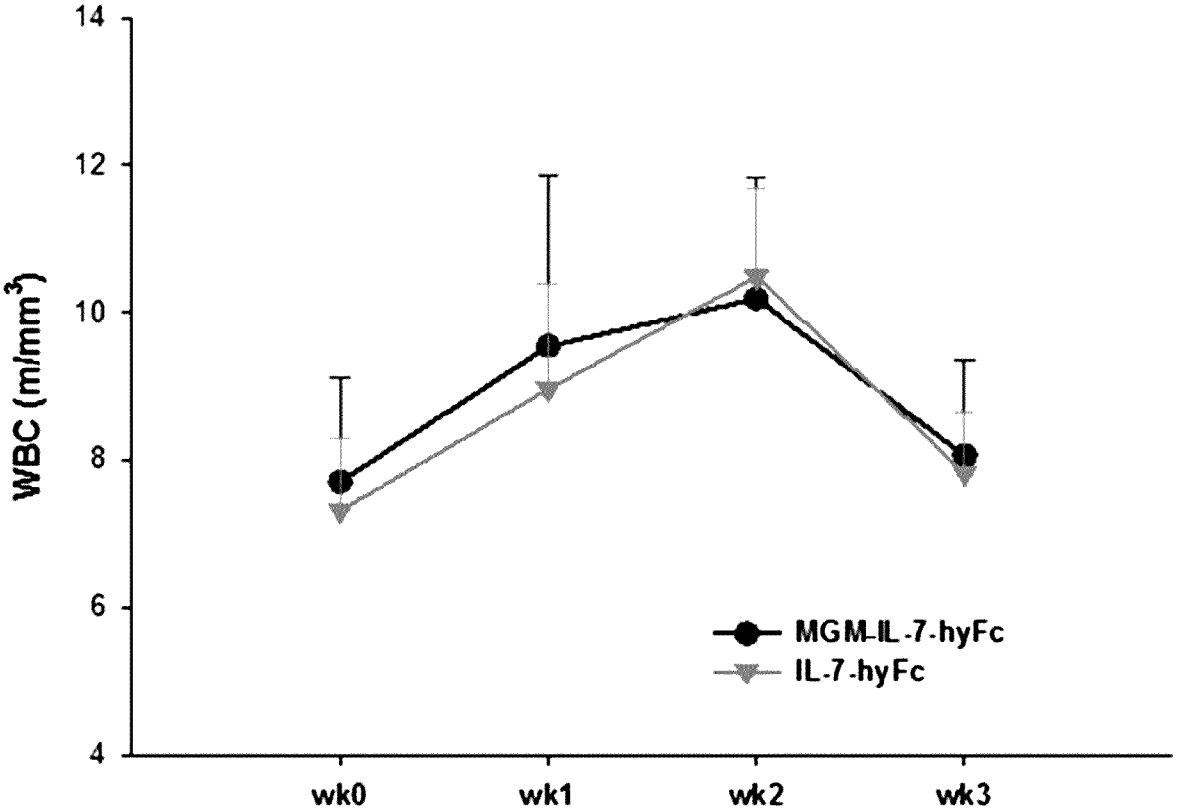
[Fig. 6]


Drug concentration in rat serum (ng/mL)
10¹
10⁰
10⁻¹
0
20
40
60
80
100
120
140
160
180
Time (h)
MGM-IL-7-hyFc
IL-7-hyFc

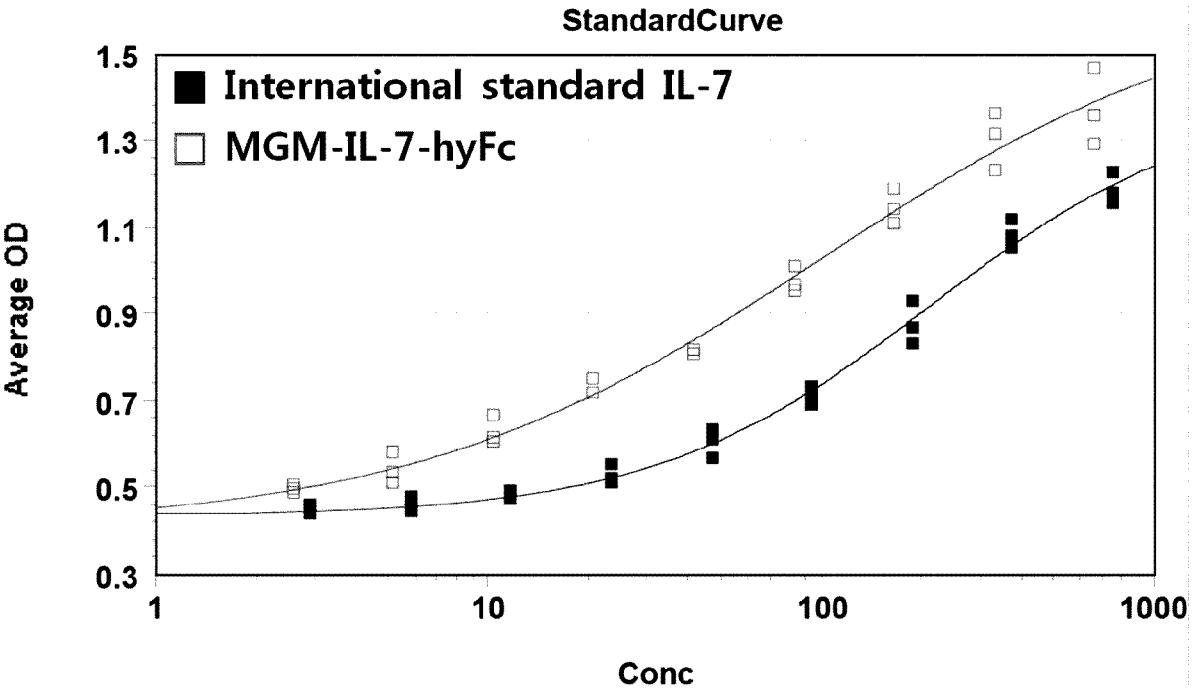
[Fig. 7]


OD value (at 450nm)
4
3
2
1
0
10¹
10²
10³
10⁴
10⁵
10⁶
Dilution fold
MGM-IL7-hyFc
IL7-hyFc

[Fig. 8]

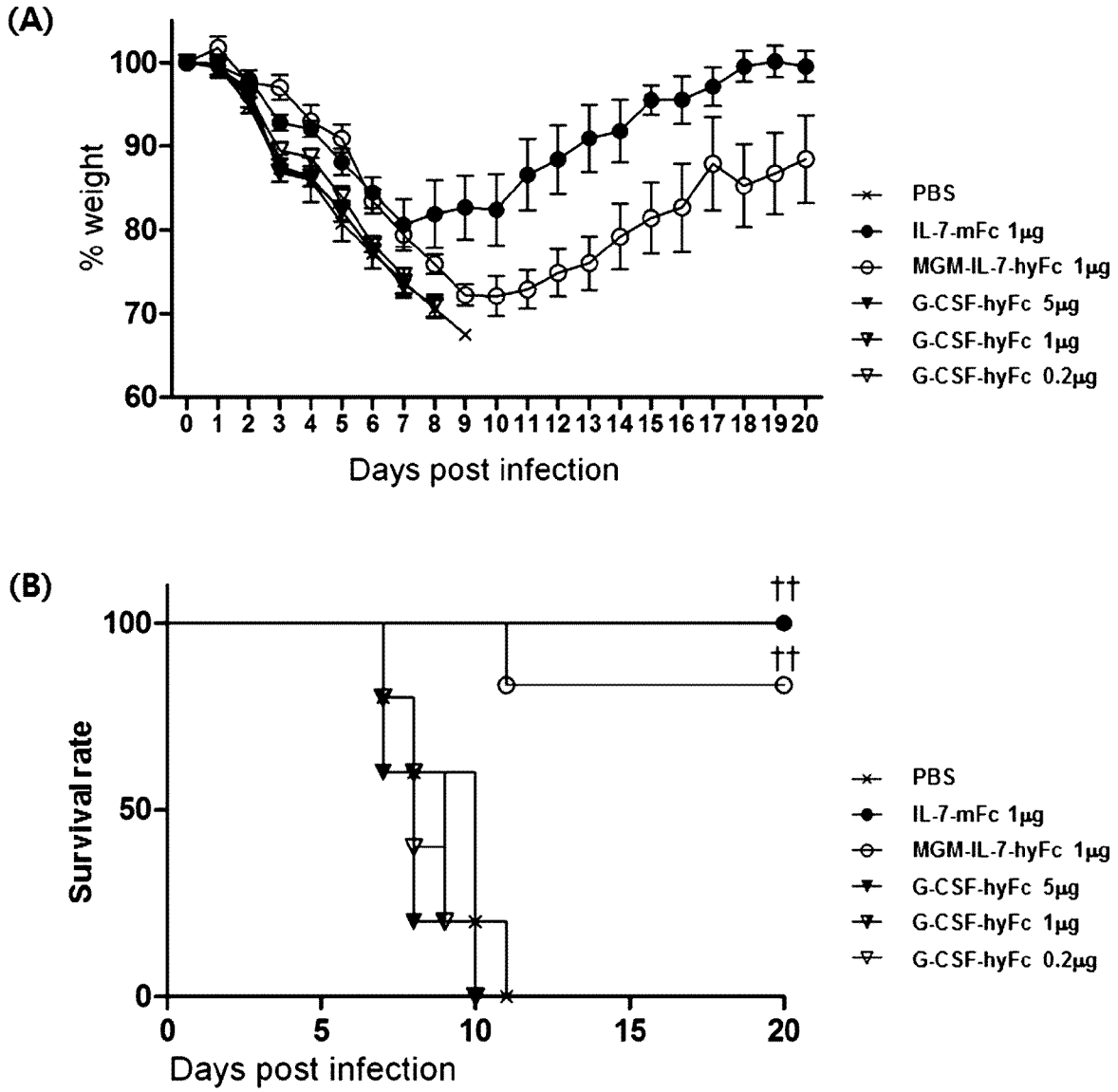


[Fig. 9]

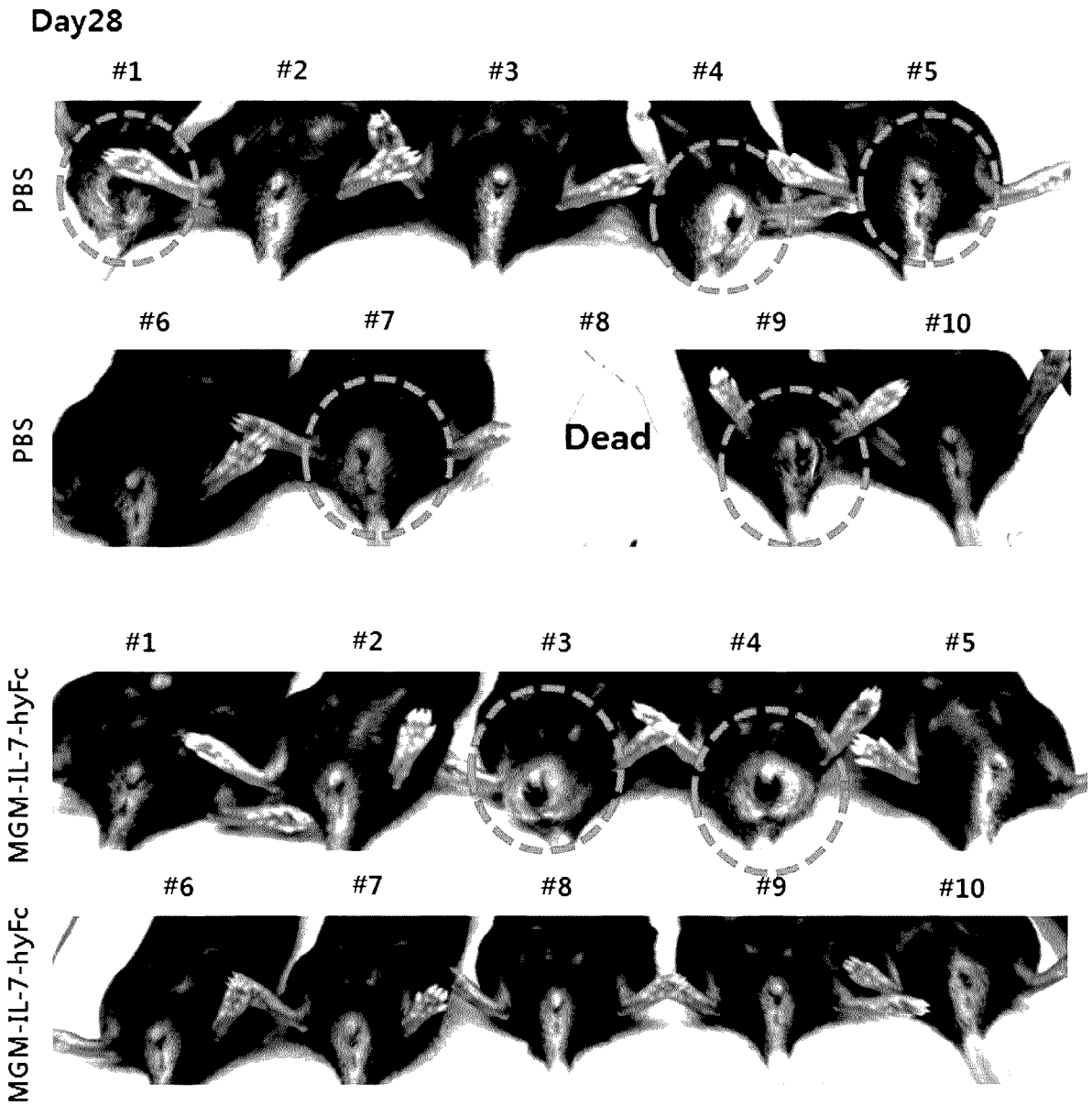


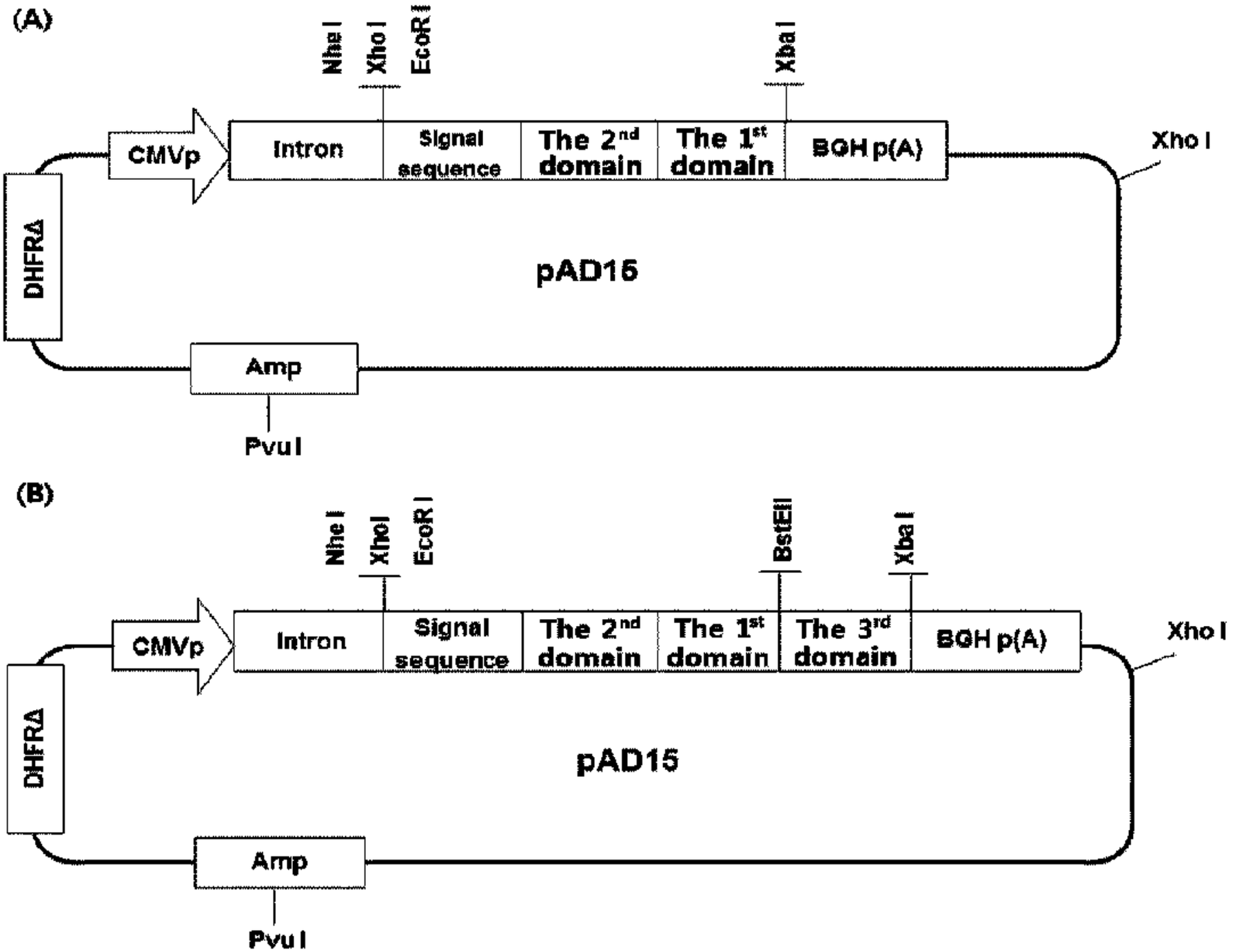
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
■ STD#1 (IS: Concentration vs Values)	0.434	1.04	211	1.4	0.991
□ Plot#1 (I7-DS-1416: Concentration vs ...	0.407	0.705	99.7	1.65	0.987

[Fig. 10]


(A)
% weight
100
90
80
70
60
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days post infection
PBS
IL-7-mFc 1μg
MGM-IL-7-hyFc 1μg
G-CSF-hyFc 5μg
G-CSF-hyFc 1μg
G-CSF-hyFc 0.2μg
(B)
Survival rate
100
50
0
0 5 10 15 20
Days post infection
††
††
PBS
IL-7-mFc 1μg
MGM-IL-7-hyFc 1μg
G-CSF-hyFc 5μg
G-CSF-hyFc 1μg
G-CSF-hyFc 0.2μg

[Fig. 11]


Day28
#1
#2
#3
#4
#5
PBS
#6
#7
#8
#9
#10
PBS
Dead
#1
#2
#3
#4
#5
MGM-IL-7-hyFc
#6
#7
#8
#9
#10
MGM-IL-7-hyFc


(A)
Nhe I
Xho I
EcoR I
Xba I
CMVp
Intron
Signal sequence
The 2nd domain
The 1st domain
BGH p(A)
Xho I
DHFRΔ
pAD15
Amp
Pvu I
(B)
Nhe I
Xho I
EcoR I
BstEII
Xba I
CMVp
Intron
Signal sequence
The 2nd domain
The 1st domain
The 3rd domain
BGH p(A)
Xho I
DHFRΔ
pAD15
Amp
Pvu I