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Fortsættes ...

(54) Benævnelse: **BACULOVIRUSVEKTORER MED EN DOBBELT VERTEBRAT- OG BACULOVIRUSPROMOTOR, DER REGULERER ET IMMUNOGENT FUSIONSGEN**

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**STRAUSS ROBERT ET AL: "Baculovirus-based vaccination vectors allow for efficient induction of immune responses against plasmodium falciparum circumsporozoite protein." MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY JAN 2007, vol. 15, no. 1, January 2007 (2007-01), pages 193-202, XP009109214 ISSN: 1525-0024**

**YOSHIDA S ET AL: "Baculovirus virions displaying Plasmodium berghei circumsporozoite protein protect mice against malaria sporozoite infection" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 316, no. 1, 10 November 2003 (2003-11-10), pages 161-170, XP004471042 ISSN: 0042-6822**

# DESCRIPTION

## TECHNICAL FIELD

[0001] The present disclosure provides a novel transfer vector, a recombinant baculovirus obtained by homologous recombination of the transfer vector and a baculovirus DNA, and methods for production thereof.

[0002] The present disclosure further provides pharmaceuticals (e.g., vaccines, and preventive or therapeutic drugs for infectious diseases such as malaria and influenza) containing the recombinant baculovirus as an active ingredient.

## BACKGROUND ART

[0003] Baculovirus has been used as a vector in methods of industrially producing a desired protein using insect cells. In recent years, it has been found that baculovirus can introduce a foreign gene not only into insect cells but also into mammalian cells, and the possibility of their use as a vector for introducing a therapeutic gene has been found. Patent Document 1 discloses a recombinant baculovirus expression vector containing multiple independent promoters comprising a promoter derived from an early gene of baculovirus, which has a DNA region containing a gene encoding a viral non-structural protein, and a promoter derived from a late gene of baculovirus, which has a DNA region containing a gene encoding a viral structural protein.

[0004] Patent Document 2 discloses a method comprising introducing into mammalian cells a non-mammalian DNA viral vector containing multiple independent promoters controlled to express a desired foreign gene linked to the promoters; and expressing the foreign gene in the mammalian cells.

[0005] Further, Patent Document 3 discloses a method of producing a protein with gene recombination technology using a baculovirus. The method comprises expressing a fusion gene obtained by linking the gp64 gene of baculovirus to a gene encoding a desired protein to produce the desired protein in a form fused with viral particles, collecting the viral particles fused with the desired protein, and cleaving the desired protein from the viral particles to collect the protein.

[0006] With respect to a baculovirus expression system, Patent Document 4 discloses a recombinant baculovirus expression vector having multiple independent promoters containing a first nucleic acid sequence encoding a detection marker, which is linked, in a form capable of functioning, to a first promoter that is active in host cells and inactive in non-acceptable cells, and a second nucleic acid sequence containing a foreign nucleic acid sequence, which is linked, in a form capable of functioning, to a second promoter that is active in non-acceptable cells.

[0007] Patent Document 5 discloses that an influenza virus hemagglutinin (HA) antigen-expressing recombinant baculovirus vector linked to a CAG promoter derived from chicken  $\beta$  actin has influenza virus infection-preventing effects and is therefore useful as a vaccine formulation.

[0008] Patent Document 6 discloses a method of producing a baculovirus vector comprising the step of co-transfecting into insect cells a plasmid containing a baculovirus promoter and a mammalian cell-derived promoter having linked thereto a gene encoding a protein expressible on the cell surface, or a plasmid containing two baculovirus promoters having linked thereto a gene encoding a protein expressible on the cell surface has been linked.

[0009] Patent Document 7 describes research on anti-influenza virus activity, i.e., activity against influenza virus infection, of a recombinant baculovirus having cDNA of influenza virus HA incorporated in a CAG promoter, and discloses that not only the recombinant baculovirus but also wild-type baculovirus have anti-influenza virus activity.

[0010] As shown above, various recombinant baculovirus vectors have been developed in recent years, and many attempts have been made to develop pharmaceuticals for mammals containing such a recombinant baculovirus vector as an active ingredient.

[0011] In this technical field, the development of pharmaceutical formulations, particularly vaccine formulations containing, as an active ingredient, a novel recombinant baculovirus, which is a recombinant baculovirus vector having a novel structure and being effective against malaria, influenza, tuberculosis, and like infectious diseases, or diseases such as cancer, has been desired.

Patent Document 1: Japanese Patent No. 3366328, Multiple Promoter Baculovirus Expression System and Defect Particle Products.

Patent Document 2: WO98/011243, Non-mammalian DNA Virus Having Modified Coating Protein.

Patent Document 3: JP No. 2002-235236-A, Methods of Producing Proteins.

Patent Document 4: JP No. 2003-284557-A, Novel Baculovirus-Transfecting Vector and Recombinant Baculovirus for Expression of Foreign Gene.

Patent Document 5: WO02/062381, Baculovirus Vector Vaccine.

Patent Document 6: WO04/029259, Baculovirus Vector, Method of Producing Baculovirus Vector, and Method of Introducing Gene.

Patent Document 7: JP No. 2005-15346-A, Baculovirus-containing Anti-viral Agent.

Patent Document 8: EP1983047 (2008-10-22)

**[0012]** Available only as Article 54(3) EPC prior art.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

### **[0013]**

FIG. 1 shows the test results of the expression of vaccine antigens from recombinant baculoviruses of the present invention in insect cells in Example 3.

Detected by gp64 Monoclonal Antibody (AcV5)

Lane 1: AcNPV-WT

Lane 2: AcNPV-CAP-PfCSP

Lane 3: AcNPV-CAP-HA1/Anhui

Lane 4: AcNPV-CAP-HA1/Vietnam

FIG. 2 (A) shows a Western blotting analysis showing the expression of the CSP gene (PfCSP) of human malaria in viral particles of recombinant baculoviruses produced from recombinant transfer vectors.

Detected by Anti-PfCSP Monoclonal Antibody (2A10)

Lane 1: AcNPV-CAP-PfCSP

Lane 2: AcNPV-CAP-PfCSP/272

Lane 3: AcNPV-CAP-PfCSP/467

FIG. 2 (B) shows a Western blotting analysis showing the expression of H5N1/HA1 gene in viral particles of recombinant baculoviruses produced from recombinant transfer vectors.

Detected by H5N1/Vietnam Rabbit Polyclonal Antibody (IT-003-005)

Lane 1: AcNPV-WT (Cell Lysate)

Lane 2: AcNPV-CAP-HA1/Anhui (Cell Lysate)

Lane 3: AcNPV-CAP-HA1/Vietnam (Cell Lysate)

Lane 4: AcNPV-WT (Virion)

Lane 5: AcNPV-CAP-HA1/Vietnam (Virion)

Lane 6: Purified H5N1/Anhui Antigen (IT-003-0053p)

FIG. 3 shows HepG2 cells stained with a fluorescence-labeled antibody, which indicates that an antigen has been expressed by a recombinant baculovirus containing a fusion gene of PfMSP1 gene and PfCSP gene in the HepG2 cells. The results of FIG. 3 (A) confirmed that a PfCSP antigen has been expressed. The results of FIG. 3 (B) confirmed that a PfMSP-1<sub>19</sub> antigen has been expressed.

FIG. 4 shows the results of measurement of antibody titers obtained in Example 6.

FIG. 5 shows the transfer vectors of the present invention.

## **DISCLOSURE OF THE INVENTION**

### **PROBLEMS TO BE SOLVED BY THE INVENTION**

**[0014]** An object of the present disclosure is to provide a novel recombinant transfer vector, a recombinant baculovirus obtained by homologous recombination of the recombinant transfer vector and a baculovirus DNA, and methods for production thereof. Another object of the present invention is to provide a pharmaceutical preparation as claimed, particularly a vaccine formulation containing the recombinant baculovirus as an active ingredient.

### **MEANS FOR SOLVING THE PROBLEMS**

**[0015]** The present inventors found a transfer vector having a novel structure capable of expressing a fusion protein of a protein having a desired immunogenicity or a partial protein thereof with a viral structural protein in insect cells and in vertebrate (particularly mammalian and avian) cells; and a recombinant baculovirus obtained by homologous recombination of the transfer vector and a baculovirus DNA. Using the obtained recombinant baculovirus, the present inventors carried out extensive research on pharmaceuticals containing as an active ingredient a recombinant baculovirus having infectious disease-preventive and therapeutic effects. As a result, the inventors found that the obtained recombinant baculovirus has the desired pharmaceutical effects.

**[0016]** Thus, the inventors of the present invention found a recombinant transfer vector having a novel structure, a recombinant baculovirus obtained by homologous recombination of the transfer vector and a baculovirus DNA, and methods for the production thereof, and ascertained that the recombinant baculovirus itself is useful as a pharmaceutical capable of expressing a protein having the desired immunogenicity in target cells, and as a pharmaceutical for preventing infectious diseases such as malaria and influenza. The present invention has been accomplished, based on these findings.

**[0017]** The present invention provides the inventions shown in the following items 1 to 2:

1. [1] A pharmaceutical composition comprising a recombinant *Autographa californica* nucleopolyhedrosis virus (AcNPV) as an active agent and, optionally, a pharmaceutically acceptable carrier, wherein the recombinant AcNPV contains a gene structure having a fusion gene of
  1. (A) a gene encoding an amino acid sequence consisting of amino acids 19-373 of SEQ ID NO:70 (*Plasmodium falciparum* CircumSporozoite Protein; PfCSP), and
  2. (B) a gene encoding an amino acid sequence consisting of amino acids 21-512 of gp64 viral particle protein (gp64) encoded in the AcNPV genome (GenBank Accession No. L22858) under the control of a dual promoter comprising Polyhedrin promoter and CAG promoter linked to each other (CAP).
2. [2] A pharmaceutical composition of [1], which is a malaria vaccine.

**[0018]** Also disclosed herein are:

Item 1. A transfer vector which is any one of pCAP-PfCSP, pCAP-PfCSP/272, pCAP-PfCSP/467, pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, pCAP-PfCSP(A361E)/467, pCAP-PfCSP-76, pCAP-PfCSP-76/467, pCAP-PfCSP+209, pCAP-PfCSP+209/467, pCAP-PfCSP+76/209, pCAP-PfCSP+76/209/467, pCAP-HA1/Anhui, pCAP-HAI/Anhui/272, pCAP-HA1/Anhui/467,

pCAP-HA1/Vietnam, pCAP-HAI/Vietnam/51, pCAP-HAI/Vietnam/101, pCAP-HA1/Vietnam/154, pCAP-HAI/Vietnam/201, pCAP-HA1/Vietnam/272, pCAP-HA1/Vietnam/467, pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, pCAP-AH/520/467, pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, pCAP-VN/520/467, pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, pCAP-CO/205/467, pCA39-HA1/Anhui, pCA64-HA1/Anhui, pCA39-PfCSP(A361E), pCA64-PfCSP(A361E), pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76/VSV, pCAP-CO/205/VSV, pDual-Pfs25-PfCSP-gp64, and pDual-PfMSP1-PfCSP-gp64.

Item 2. A recombinant baculovirus which is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

Item 3. A composition for infectious diseases comprising the recombinant baculovirus of item 2 as an active ingredient.

Item 4. A composition for infectious diseases comprising the recombinant baculovirus of item 2 as an active ingredient, the composition being administered by the intramuscular, respiratory, or nasal route.

Item 5. A vaccine comprising the recombinant baculovirus of item 2 as an active ingredient.

Item 6. A vaccine comprising the recombinant baculovirus of item 3 as an active ingredient, the composition being administered by the intramuscular, respiratory, or nasal route.

Item 7. A therapeutic or preventive agent for influenza virus infection, comprising as an active ingredient any one of AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HAI/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CA39-HA1/Anhui, and AcNPV-CA64-HA1/Anhui.

Item 8. A therapeutic or preventive agent for influenza virus infection according to item 7, which is administered by the intramuscular, respiratory, or nasal route.

Item 9. A vaccine against influenza virus infection, comprising as an active ingredient any one of AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CA39-HAI/Anhui, and AcNPV-CA64-HA1/Anhui.

Item 10. A vaccine against influenza virus infection according to item 7, which is administered by the intramuscular, respiratory, or nasal route.

Item 11. A therapeutic or preventive agent for human malaria infection, comprising as an active ingredient any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

Item 12. A therapeutic or preventive agent for human malaria infection according to item 11, which is administered by the intramuscular, respiratory, or nasal route.

Item 13. A therapeutic or preventive agent for human malaria infection, comprising as an active ingredient any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

Item 14. A therapeutic or preventive agent for human malaria infection according to item 13, which is administered by the intramuscular, respiratory, or nasal route.

Item 15. A method of preventing malaria or influenza infection or of treating malaria or influenza, comprising administering to a subject an effective amount of the recombinant baculovirus of item 2, the composition for infectious diseases of item 3 or 4, or the vaccine of item 5, 6, 9, 10, 13 or 14.

Item 16. A method according to item 15, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject as a liposomal formulation.

Item 17. A method according to item 15, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject by the intramuscular, respiratory, or nasal route.

Item 18. A method according to item 16, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject by the intramuscular, respiratory, or nasal route.

Item 19. A method of immunostimulation comprising administering to a subject an effective amount of the recombinant baculovirus of item 2, the composition for infectious diseases of item 3 or 4, or the vaccine of item 5, 6, 9, 10, 13 or 14.

Item 20. A method according to item 19, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject as a liposomal formulation.

Item 21. A method according to item 19, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject by the intramuscular, respiratory, or nasal route.

Item 22. A method according to item 20, wherein the recombinant baculovirus, composition, or vaccine is administered to the subject by the intramuscular, respiratory, or nasal route.

## **EFFECT OF THE INVENTION**

**[0019]** According to the present disclosure, a novel recombinant transfer vector, a recombinant baculovirus obtained by homologous recombination of the recombinant transfer vector and a baculovirus DNA, and methods for production thereof are provided. Pharmaceuticals containing the recombinant baculovirus of the present disclosure as an active ingredient are useful as therapeutic or preventive drugs for infectious diseases such as malaria and influenza, or as cellular medicine and vaccine formulations.

## **BEST MODE FOR CARRYING OUT THE INVENTION**

**[0020]** The abbreviations used for the amino acids, peptides, base sequences, and nucleic acids in the present specification are based on the abbreviations specified in the IUPAC-IUB Communication on Biochemical Nomenclature, Eur. J. Biochem., 138: 9 (1984) and "Guideline for Preparing Specifications Including Base Sequences and Amino Acid Sequences" (Patent Office), and those commonly used in this technical field. In the present specification, the DNA molecule may include not only double strand DNA but also single strand DNA, i.e., a sense chain and an antisense chain constituting the double strand DNA, and is not limited to a full length thereof. The polynucleotide (DNA molecule) encoding the immunogenic foreign gene of the present disclosure encompasses double strand DNA containing genomic DNA, single strand DNA (sense chain) containing cDNA, and single strand DNA (antisense chain) having a sequence complementary to the sense chain, synthetic DNA, and fragments thereof, unless

otherwise mentioned.

**[0021]** The polynucleotide or DNA molecule used herein is not limited in the functional region, and may include at least one of an expression suppression region, a coding region, a leader sequence, an exon, and an intron.

**[0022]** Further, examples of the polynucleotide include RNA and DNA. The polypeptide containing a specific amino acid sequence and the polynucleotide containing a specific DNA sequence include fragments, homologs, derivatives, and mutants of the polynucleotide.

**[0023]** Examples of mutants of the polynucleotide, such as mutant DNA, include naturally occurring allelic mutants; artificial mutants; and mutants having deletion, substitution, addition, and/or insertion. However, it should be understood that such mutants encode polypeptides having substantially the same function as the polypeptide encoded by the original non-mutated polynucleotide.

**[0024]** In the present invention, the transfer vector refers to a plasmid for producing a recombinant baculovirus having a structure in which a fusion gene, formed by linking at least one gene encoding a protein capable of being a component of viral particles to at least one immunogenic foreign gene, is incorporated downstream of a dual promoter comprising two linked promoters, i.e., one vertebrate promoter (e.g., a mammalian promoter or an avian promoter) and one baculovirus promoter.

**[0025]** In one preferable embodiment of the invention, the immunogenic foreign gene is located downstream of the dual promoter and upstream of the gene encoding a protein capable of being a component of viral particles. The recombinant baculovirus of the present disclosure is used as an active ingredient of pharmaceuticals or vaccines for vertebrates. Examples of vertebrates include mammals such as humans, cattle, horses, swine, sheep, goats, monkeys, mice, dogs, and cats. Examples of birds include chickens, quail, geese, wild ducks, pigeons, turkeys, guinea fowls, and parrots.

**[0026]** In one embodiment, the present disclosure provides a transfer vector containing a novel structure having incorporated therein a fusion gene of one immunogenic foreign gene and a gene encoding a viral membrane protein that can be expressed in insect cells under the control of a dual promoter comprising one vertebrate promoter and one baculovirus promoter linked to each other. Co-transfection of this transfer vector with a baculovirus DNA into insect cells induces a homologous recombination and thereby produces a recombinant baculovirus having incorporated therein the fusion gene that is expressed under the control of a baculovirus promoter in insect cells and that can produce a fusion protein capable of being a component of budded viral particles.

**[0027]** In the present invention, when the recombinant baculovirus is administered to a vertebrate, a fusion protein of a protein capable of being a component of budded viral particles with an immunogenic protein seems to function as a component vaccine. Further, the recombinant baculovirus administered to the vertebrate enters the vertebrate cell, and a fusion antigen with the desired immunogenic foreign antigen is produced from the viral genome in the vertebrate cell, and functions as a DNA vaccine.

**[0028]** Thus, for example, when the recombinant baculovirus of the present disclosure is administered to a mammal, a fusion protein of a protein capable of being a component of viral particles with an immunogenic protein is presented as an antigen on the surface of virus particles, and a fusion protein of a protein capable of being a component of viral particles with an immunogenic protein is produced in the mammalian cell, and seems to function as a preventive or therapeutic agent for infectious diseases, such as viral, protozoan, and bacterial infection, due to its immunopotentiative action.

**[0029]** The baculovirus DNA to be co-transfected with the transfer vector may be a wild type, mutant, or recombinant baculovirus DNA. Examples of host cells to be co-transfected include insect cells such as cells of *Spodoptera frugiperda*.

**[0030]** In the present disclosure, the immunogenic foreign gene refers to a gene encoding the amino acid sequence of an antigenic protein used as an immunogen in immunotherapy, such as vaccine therapy, for the prevention and treatment of infectious diseases, such as malaria and influenza. Specific examples thereof include genes encoding the amino acid sequences of proteins, such as malaria antigen and influenza virus antigen.

**[0031]** The "foreign" gene as used herein refers to a gene introduced from the outside. Even if the same gene is present in the cell, the gene introduced from the outside is referred to as the "foreign" gene.

**[0032]** In the present disclosure, the gene encoding the amino acid sequence of a protein used as the immunogen is not particularly limited, as long as the gene is capable of encoding the amino acid sequence of an antigenic protein having immunogenicity against a substance that causes diseases such as infectious diseases. Examples of genes encoding the amino

acid sequence of an antigenic protein having immunogenicity are as follows.

**[0033]** Examples of genes encoding the amino acid sequence of a malaria antigen include genes encoding the amino acid sequences of sporozoite surface antigen CSP (Circumsporozoite Protein) of malaria parasites, merozoite surface membrane protein MSP1 (Merozoite Surface Protein 1), malaria S antigen secreted from erythrocytes infected with malaria, PfEMP1 protein present in the knobs of erythrocytes infected with malaria, SERA protein, TRAMP protein, AMA1 protein, and like proteins.

**[0034]** Examples of genes encoding the amino acid sequences of influenza virus antigens include genes encoding the amino acid sequences of HA antigen (hemagglutinin antigen), NA antigen (neuraminidase antigen), M2 antigen (matrix protein antigen), NP antigen (nucleoprotein antigen), and like proteins.

**[0035]** Of the vertebrate genes, examples of mammalian genes include genes encoding the amino acid sequences of antigenic proteins of infectious diseases in humans, cattle, horses, swine, sheep, monkeys, mice, dogs, and cats. Examples of avian genes include antigen genes (e.g., avian influenza HA antigen) of infectious diseases in chickens, wild ducks, pigeons, turkeys, guinea fowls, and parrots.

**[0036]** Pathogen genes whose association with infectious diseases in mammals and birds as mentioned above has been reported are easily available from institutions storing registered public data on pathogen genes, such as Genbank.

**[0037]** The present disclosure can provide a transfer vector having such an immunogenic foreign gene, and a recombinant baculovirus obtained by homologous recombination of the transfer vector. Further, the present invention can provide a pharmaceutical composition as claimed containing as an active ingredient the recombinant baculovirus having the immunogenic foreign gene, and a vaccine formulation of the pharmaceutical composition.

**[0038]** The baculovirus used in the present disclosure refers to insect pathogen viruses that cause infection in insects, which are one group (*Baculoviridae*) of DNA viruses having a cyclic double strand DNA as a gene. Among these, one group of the viruses referred to as the nucleopolyhedrosis virus (NPV) produce an inclusion body, referred to as a polyhedron, in the nucleus of infected cells in the late phase of infection. Even if a foreign gene to be expressed is inserted in place of the polyhedrin gene, virus infection and growth sufficiently occur to produce a large amount of the desired foreign gene product. Therefore, this virus has been used for the production of desired proteins in recent years.

**[0039]** Examples of the baculovirus used in the present disclosure include *Autographa californica nucleopolyhedrosis* virus: AcNPV, *Bombyx mori nucleopolyhedrosis* virus: BmNPV, *Orgyia pseudotsugata nucleopolyhedrosis* virus: OpNPV, and *Lymantria disper nucleopolyhedrosis* virus: LdNPV.

**[0040]** The baculovirus DNA may be any DNA that can be homologously recombined with the transfer vector of the present disclosure. More specifically, the viral gene of the baculovirus DNA that can be homologously recombined with the transfer vector of the present disclosure is as huge as 130 kbp in size, and an immunogenic foreign gene of 15 kbp or more can be inserted therein. Because the baculovirus gene itself is scarcely expressed in vertebrate cells, there is almost no need to consider its cytotoxicity. Thus, it is thought that no harmful immune response is induced.

#### **(1) Transfer Vector and Production of the Transfer Vector used in the Present Invention**

##### **Production of Immunogenic Foreign Gene DNA**

**[0041]** The immunogenic foreign gene DNA capable of being fused to the viral gene, which is one of the components of the baculovirus transfer vector, can be easily produced or obtained by synthesis based on the nucleic acid sequence information of the polynucleotide encoding the amino acid sequence of an antigenic protein having the desired immunogenicity disclosed in this specification, or directly synthesizing the DNA corresponding to the nucleic acid sequence of the coding region of the immunogenic foreign gene, based on the nucleic acid sequence information of the immunogenic foreign gene (chemical DNA synthesis method). General gene engineering techniques can be used for this production (see, for example, "Molecular Cloning" 2d Ed, Cold Spring Harbor Lab. Press (1989); Zoku Seikagaku Jikken Kouza, "Idenshi Kenkyuho I, II, III", edited by the Japanese Biochemistry Society, 1986).

**[0042]** Examples of methods for synthesizing the DNA include chemical synthesis means, such as the phosphate triester method

and the phosphate amidite method (J. Am. Chem. Soc., 89, 4801 (1967); *ibid.*, 91, 3350 (1969); Science, 150, 178 (1968); Tetrahedron Lett., 22, 1859 (1981); *ibid.*, 24, 245 (1983)) and combination methods thereof. The DNA can also be chemically synthesized by the phosphoramidite method or the triester method, or synthesized by using a commercially available automatic oligonucleotide synthesizer. A double strand fragment can be obtained by synthesizing a complementary strand and annealing the complementary strand with a chemically synthesized single strand under appropriate conditions, or adding the complementary strand with appropriate primer sequences to a chemically synthesized single strand using a DNA polymerase.

**[0043]** Specific examples of the immunogenic foreign gene DNA produced in the present disclosure include DNA comprising a DNA sequence encoding the amino acid sequence of a malaria antigen protein, and a DNA sequence encoding the amino acid sequence of an influenza virus antigen protein.

**[0044]** The DNA used in the present disclosure is not limited to a full-length DNA sequence encoding the amino acid sequence of the polypeptide of an antigenic protein having the immunogenicity, and may be a DNA sequence encoding a partial sequence thereof, as long as the protein of the amino acid sequence encoded by the DNA sequence has the immunogenicity.

**[0045]** The DNA of the immunogenic foreign gene used in the present disclosure is not limited to DNA molecules having such a specific DNA sequence, and may be DNA molecules having a DNA sequence produced by suitably selecting codons for each amino acid residue. The choice of codons can be made in accordance with a standard method, for example, by considering the frequency of codon use in the host used (Nucleic Acids Res., 9, 43(1981)).

**[0046]** The DNA of the immunogenic foreign gene used in the present invention can be produced by gene engineering techniques, more specifically by preparing a cDNA library from an appropriate origin that expresses the DNA of an immunogenic foreign gene in accordance with a standard method, and selecting a desired clone from the library using an appropriate probe or antibody against the expressed product, which is specific to the immunogenic foreign gene (see, for example, Proc. Natl. Acad. Sci., USA., 78, 6613 (1981); Science, 222, 778 (1983)).

**[0047]** Examples of the genomic DNA origin include various cells, tissues, and cultured cells derived therefrom, which express the DNA of the immunogenic foreign gene. Extracts of erythrocytes infected with malaria parasites, and extracts of cells infected with influenza viruses are particularly preferable. The extraction and separation of total DNA and RNA from the origin, separation and purification of mRNA, and the production and cloning of cDNA can be performed in accordance with standard methods.

**[0048]** As described above, the DNA of the immunogenic foreign gene can be produced by using the cDNA library of each immunogen prepared by extraction, separation and purification of the mRNA of the immunogenic tissue or cells from the above-mentioned extract. The DNA of the immunogenic foreign gene can also be produced by using a phage library prepared by extracting the mRNA of each immunogen, adding poly A to the RNA, collecting the poly A-added RNA, producing cDNA using a reverse transcriptase, adding restriction enzyme sites to both ends of the cDNA, and incorporating the cDNA into a phage.

**[0049]** The method of screening the immunogenic foreign gene DNA from the cDNA library is not particularly limited, and can be performed in accordance with a usual method. Specific examples of such methods include a method of selecting a corresponding cDNA clone by immunological screening using a specific antibody (e.g., anti-malaria antibody, anti-influenza virus antibody, etc.) against the protein produced by the cDNA; a plaque hybridization method using a probe selectively binding to the target DNA sequence; a colony hybridization method; and combinations thereof.

**[0050]** The probe used in such hybridization methods is usually a DNA fragment chemically synthesized based on the information on the DNA sequence of the immunogenic foreign gene. The DNA sequences of the immunogenic foreign gene already obtained or fragments thereof can also be advantageously used as the probe. A sense primer and an antisense primer designed based on the DNA sequence information of the immunogenic foreign gene can also be used as the probe for the screening.

**[0051]** The DNA (nucleotides) used as the probe is a partial DNA (nucleotides) corresponding to the DNA sequence of an immunogenic foreign gene. The DNA (nucleotides) has an at least 15 consecutive DNA, preferably at least 20 consecutive DNA, and more preferably at least 30 consecutive DNA sequence. A positive clone itself for producing the DNA as mentioned above can also be used as the probe.

**[0052]** To obtain the DNA of the immunogenic foreign gene, a DNA/RNA amplification method by PCR (Science, 230, 1350 (1985)) is preferably used. In particular, when a full length cDNA is hardly obtained from the library, the RACE method (Rapid amplification of cDNA ends; Jikken Igaku 12(6), 35(1994)), particularly the 5'-RACE method (M. A. Frohman, et al., Proc. Natl. Acad. Sci., USA., 8, 8998(1988)) is preferably used.

**[0053]** The primers used for PCR can be designed, based on the DNA sequence information of the immunogenic foreign gene, and synthesized in accordance with standard methods. As the primers, DNA portions (SP6 promoter/primer and T7 terminator/primer) added to both ends of a vector plasmid having the DNA of the immunogenic foreign gene incorporated therein can also be used, as described in the Examples below.

**[0054]** The isolation/purification of the DNA/RNA fragment amplified by PCR can be performed in accordance with standard methods, for example, by gel electrophoresis.

**[0055]** The DNA sequence of the DNA of the immunogenic foreign gene thus obtained or various DNA fragments thereof can be determined in accordance with standard methods, for example, by the dideoxy method (Proc. Natl. Acad. Sci., USA., 74, 5463(1977)) or Maxam-Gilbert method (Methods in Enzymology, 65, 499(1980)), or by simply using a commercially available sequencing kit.

**[0056]** The gene encoding the amino acids of a protein capable of being a component of viral particles may be any gene that can encode a protein that is formed into a fusion protein with an immunogenic foreign gene as mentioned above in target cells and can be expressed as a protein capable of being a component of viral particles in insect cells.

**[0057]** Examples of the gene encoding the amino acids of a protein capable of being a component of viral particles include genes of gp64 protein (GenBank Accession No. L22858), vesicular stomatitis virus glycoprotein G (VSVG: GenBank Accession No. J02428), herpes simplex virus glycoprotein (KOS: GenBank Accession No. K01760), human immunodeficiency virus type I gp120 (GenBank Accession No. U47783), human respiratory syncytial virus membrane glycoprotein (GenBank Accession No. M86651), influenza A virus hemagglutinin protein (GenBank Accession No. U38242), and genes of envelope proteins of viruses closely related to baculovirus.

Amino acid sequence of gp64 viral particle protein (gp64) encoded by nucleotides 108179 to 109717 in the AcNPV genome (GenBank Accession No. L22858):

```
MVSAIVLYVLLAAAHSAFAAEHCNAQMKTPYKIKNLDITPPKETLQKDVETIVETDYNENVIIGYKG
YYQAYAYNGGSLDPNTRVEETMKTFLNVGKEDLLMWSIRQQCEVGEELIDRWGSDSDDCFRDNEGRGQVW
GKELVQRQNNHFAHHTCNKSWRCGISTSKMYSRLEQDDTDECQVYILDAEGNFINVTVDTVLHRDGV
MILKQKSTFTTRQIKAAACLLIKDDKNNPESVTRHCLIDNDIYDLSKNTWNCNFKRICKRVEHRVKKRP
PTWRHNVRAKYTEGDTATRGDLMIHQEELMYENDLLKMNIELMHAHINKLNMLHDLIVSVAKVDERLIG
NLMNNSVSSFTLSDDTFLMPCNTNPPAHTSNCYNNISYKEGRWVANTDSSQCIDFSNYKELAIIDDVFEW
IPTIGNTTYHDSWKDASGWSFTAQQKSNLITMENTKFGVGTSLSDITSMAEGLAAKLTSPMFGHVVN
FVILVILFLYCMIRNRNQY
```

Amino acid 21-512 of gp64 encoded in the AcNPV genome (GenBank Accession No. L22858):

```
AEHCNAQMKTPYKIKNLDITPPKETLQKDVETIVETDYNENVIIGYKGYQAYAYNGGSLDPNTRVEE
TMKTFLNVGKEDLLMWSIRQQCEVGEELIDRWGSDSDDCFRDNEGRGQVWKGKELVQRQNNHFAHHTCNK
SWRCGISTSKMYSRLEQDDTDECQVYILDAEGNFINVTVDTVLHRDGVSMILKQKSTFTTRQIKAAACLL
IKDDKNNPESVTRHCLIDNDIYDLSKNTWNCNFKRICKRVEHRVKKRPPTWRHNVRAKYTEGDTATKG
DLMIHQEELMYENDLLKMNIELMHAHINKLNMLHDLIVSVAKVDERLIGNLMNNSVSSFTLSDDTFLM
PCTNPPAHTSNCYNNISYKEGRWVANTDSSQCIDFSNYKELAIIDDVFEWIPPTIGNTTYHDSWKDASGWS
FIAQQKSNLITMENTKFGVGTSLSDITSMAEGLAAKLTSPMFGHVNVFVILVILFLYCMIRNRNQY
```

**[0058]** In the present invention, the gp64 gene described in Examples below is used.

**[0059]** The DNA of the gene encoding the amino acids of a protein capable of being a component of viral particles can be easily produced or obtained by synthesis based on the nucleic acid sequence information of the polynucleotide encoding the amino acid sequence of the polypeptide of the gene encoding the amino acids of the target protein capable of being a component of viral particles; or by directly synthesizing the DNA corresponding to the nucleotide sequence encoding the amino acid sequence, based on the amino acid sequence information of the gene encoding the amino acids of the protein capable of being a component of viral particles (chemical DNA synthesis), as in the case of the production of the DNA of the immunogenic foreign gene.

**[0060]** The DNA sequence corresponding to the nucleic acid sequence encoding the amino acids of a protein capable of being a component of viral particles is not limited to a full-length coding region, and may be DNA containing a partial DNA sequence thereof.

**[0061]** As in the case of the production of the DNA molecule of the immunogenic foreign gene, the DNA of the gene encoding the amino acids of a protein capable of being a component of viral particles can be produced by general gene engineering techniques (see, for example, Molecular Cloning 2d Ed, Cold Spring Harbor Lab. Press (1989); Zoku Seikagaku Jikken Kouza, "Idenshi Kenkyuho I, II, III", edited by the Japanese Biochemistry Society, 1986).

[0062] In the present invention, commercially available vector plasmids into which a part of the promoter that controls the expression of the immunogenic foreign gene described later has been incorporated and a (partial) gene encoding the amino acids of a protein capable of being a component of viral particles has been introduced can also be used.

#### **Vertebrate Promoters**

[0063] Examples of the vertebrate promoter (promoter capable of functioning in vertebrate cells) that is one of the components of the transfer vector used in the present disclosure include mammalian promoters and avian promoters.

#### **Mammalian Promoters**

[0064] Examples of the mammalian promoter (promoter capable of functioning in mammalian cells) that is one of the components of the transfer vector used in the present disclosure include cytomegalovirus promoters, SV40 promoters, retrovirus promoters, metallothionein promoters, heat shock protein promoters, CAG promoters, elongation factor 1 $\alpha$  promoters, actin promoters, ubiquitin promoters, albumin promoters, and MHC promoters.

#### **Avian Promoters**

[0065] Examples of the avian promoter include  $\beta$  actin promoters, heat shock protein promoters, elongation factor promoters, ubiquitin promoters, and albumin promoters.

#### **Baculovirus Promoters**

[0066] Examples of the baculovirus promoter that is one of the components of the baculovirus transfer vector used in the present disclosure include polyhedrin promoter, p10 promoter, ie1 promoter, p35 promoter, vp39 promoter, and gp64 promoter.

#### **Production of Recombinant Transfer Vector**

[0067] The present disclosure relates to a novel transfer vector having a structure capable of expressing the desired immunogenic foreign gene as an antigenic protein in both insect cells and vertebrate cells, particularly mammalian cells. The novel transfer vector produced in the present invention has a structure in which a DNA sequence encoding the amino acid sequence of the desired immunogenic protein and a DNA sequence encoding the amino acid sequence of a protein capable of being a component of viral particles are linked downstream of linked promoters comprising one vertebrate promoter, particularly a mammalian promoter, and one baculovirus promoter. The DNA regions containing the DNA sequences of the two promoters, i.e., one vertebrate promoter, particularly a mammalian promoter, and one baculovirus promoter, may be directly linked to each other, or an intervening DNA sequence may be present between the DNA sequences of the two promoters (provided, however, that the two promoters should have promoter activity in insect cells and in vertebrate cells, and particularly mammalian cells). The promoter region has a structure such that either of a vertebrate promoter, particularly a mammalian promoter, and a baculovirus promoter, linked to each other, may be disposed more closely to the gene to be expressed. In the Examples described later, a baculovirus promoter is disposed more closely to the gene to be expressed than a mammalian promoter.

[0068] In the above structure, the DNA sequence of the fusion gene of a gene encoding a protein capable of being a component of viral particles and a desired immunogenic foreign gene may be such that these two genes are directly linked to each other, or an intervening DNA sequence is present between the genes (provided, however, that it is necessary not to cause a frameshift of the downstream gene and the upstream gene). Preferably, the antigen-presenting domain of the protein of a foreign gene having the desired immunogenicity is fused to a protein capable of being a component of viral particles. Therefore, the protein of a foreign gene having the desired immunogenicity should not be cut off from the protein capable of being a component of viral particles, but should be used in a form fused therewith.

[0069] A fusion gene containing these two genes may be prepared in advance, and then incorporated into the vector.

Alternatively, one of the genes may first be incorporated into the vector and then the other may be incorporated into the vector to form a fusion gene in the vector.

**[0070]** To produce such a transfer vector, commercially available expression vectors already having some essential components of the transfer vector used in the present invention, i.e., a promoter region containing a vertebrate promoter, particularly a mammalian promoter, and a baculovirus promoter, and a gene region encoding the amino acid sequence of a protein capable of being a component of viral particles, may be used, and the required components can be inserted by optionally cleaving such a commercially available expression vector with restriction enzymes and incorporating other promoter to insert a fused DNA sequence of a foreign gene having the desired immunogenicity and a gene encoding the amino acid sequence of a protein capable of being a component of viral particles into the cloning region of the vector, or by inserting a foreign gene having the desired immunogenicity into the N terminus side of the DNA region of a gene encoding the amino acid sequence of a protein capable of being a component of viral particles, already incorporated in a plasmid.

**[0071]** In the present invention, the plasmid vector having a structure capable of expressing a foreign protein having the desired immunogenicity as an antigenic protein in both insect cells and vertebrate cells, particularly mammalian cells, may be produced by using a commercially available plasmid already having a partial structure thereof. The amino acid sequence of a peptide for cleaving the fusion protein with enzymes in vertebrate cells may intervene. In the transfer vector used in the present invention, an enhancer for increasing the transcription activity in vertebrate cells, particularly mammalian cells, may be disposed upstream of the two promoters, or a DNA sequence encoding the amino acid sequence of a signal peptide for facilitating extracellular secretion of the expressed protein from host cells may be bound to the gene to be fused and expressed. To terminate the transcription, a vertebrate terminator region, such as a rabbit  $\beta$  globin terminator, which is effective in vertebrate cells, may be disposed downstream of the gene to be fused and expressed.

**[0072]** A transfer vector capable of expressing a fusion gene of an immunogenic foreign gene capable of expressing the desired immunogenicity in baculovirus particles and a gene encoding the amino acid sequence of a protein capable of being a component of viral particles can be thereby produced.

**[0073]** Specific examples of the transfer vector and the method for production thereof according to the present disclosure are as shown in the Examples described later. More specifically, transfer vectors having a structure in which a CAG promoter modified from a cytomegalovirus (CMV) promoter as a vertebrate promoter, particularly a mammalian promoter, and a polyhedrin (polh) promoter, vp39 promoter, or gp64 promoter as a baculovirus promoter, are linked to each other, and a fused DNA sequence of an influenza virus antigen gene or malaria antigen gene as a foreign gene and a gp64 antigen gene as a gene encoding the amino acid sequence of a protein capable of being a component of the viral particle is inserted can be mentioned as examples of pCAP-PfCSP, pCAP-PfCSP/272, pCAP-PfCSP/467, pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, pCAP-PfCSP(A361E)/467, pCAP-PfCSP-76, pCAP-PfCSP-76/467, pCAP-PfCSP+209, pCAP-PfCSP+209/467, pCAP-PfCSP+76/209, pCAP-PfCSP+76/209/467, pCAP-HA1/Anhui, pCAP-HA1/Anhui/272, pCAP-HA1/Anhui/467, pCAP-HA1/Vietnam, pCAP-HA1/Vietnam/51, pCAP-HA1/Vietnam/101, pCAP-HA1/Vietnam/154, pCAP-HA1/Vietnam/201, pCAP-HA1/Vietnam/272, pCAP-HA1/Vietnam/467, pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, pCAP-AH/520/467, pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, pCAP-VN/520/467, pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, pCAP-CO/205/467, pCA39-HA1/Anhui, pCA64-HA1/Anhui, pCA39-PfCSP(A361E), pCA64-PfCSP(A361E), pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76/VSV, pCAP-CO/205/VSV, pDual-Pfs25-PfCSP-gp64, and pDual-PfMSP1-PfCSP-gp64.

## **(2) Production of Recombinant Baculovirus**

**[0074]** The present disclosure provides a method of producing a recombinant baculovirus comprising the step of producing a transfer vector having a structure in which a fusion gene containing at least one gene encoding a protein capable of being a component of the viral particle and at least one immunogenic foreign gene is incorporated downstream of a dual promoter consisting of two linked promoters, i.e., one vertebrate promoter and one baculovirus promoter, and the step of co-transfecting the transfer vector and a baculovirus DNA into a host cell and isolating the recombinant baculovirus.

**[0075]** In the above process of producing the recombinant baculovirus, the method of introducing the desired recombinant DNA (transfer vector) into the host cell and the method of transformation thereby are not particularly limited. Various methods that are well known and commonly used in this field can be used. For example, general genetic recombination techniques (e.g., Science, 224, 1431 (1984); Biochem. Biophys. Res. Comm., 130, 692 (1985); Proc. Natl. Acad. Sci. USA, 80, 5990 (1983)) can be used to

prepare the recombinant baculovirus. The recombinant DNA (transfer vector) can be expressed and produced with reference to Ohno et al., "Tanpaku Jikken Protocol 1 Functional Analysis, Saibo Kogaku Bessatsu, Jikken Protocol Series, 1997, Shujun-sha". The general methods used for the handling of insect cells, gene recombination, and co-transfection may be the same as well-known methods of producing a recombinant virus in insect cells (BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL, Oxford University Press, 1994).

**[0076]** The resulting recombinant baculovirus can be cultured in accordance with standard methods. A fusion product (expressed product) of the DNA of a foreign gene having the desired immunogenicity with the DNA encoding the amino acid sequence of a protein capable of being a component of viral particles used in the present invention is expressed and produced (accumulated and secreted) inside, outside, or on the cell membrane of insect cells by the culturing of the baculovirus.

**[0077]** As a medium used for the culturing, various commonly used media can be selectively used according to the host cell used. The culture can be performed under conditions suitable for the growth of the host cell.

**[0078]** More specifically, the method of producing the recombinant baculovirus comprises the step of preparing a baculovirus DNA to be subjected to homologous recombination with a transfer vector produced above, and the step of co-transfecting the transfer vector and the baculovirus DNA into insect cells, such as Sf9 cells and Sf21 cells derived from *Spodoptera frugiperda*, and Tn5 cells (High Five cells) derived from *Trichoplusia ni*, as host cells.

**[0079]** The baculovirus DNA thus produced to be subjected to homologous recombination with the transfer vector may be a wild type, mutant, or recombinant baculovirus DNA. The baculovirus DNA can enhance the probability of its homologous recombination with the transfer vector used in the present invention as long as it has a DNA structure homologous to the baculovirus-derived DNA containing DNA of the dual promoter region, and DNA of a fusion gene obtained by fusion of an immunogenic foreign gene and a gene encoding a protein capable of being a component of viral particles.

**[0080]** To induce homologous recombination, the mixing ratio of the transfer vector to the baculovirus DNA by weight is preferably about 1:1 to about 10:1.

**[0081]** After the co-transfection step of simultaneous introduction into insect cells, the cells are cultured, and viral plaques are obtained from the culture supernatant and then suspended in a medium. The virus is eluted from agar by vortex and centrifuged to yield a solution containing the recombinant virus.

**[0082]** In the above process, commercially available products may be used as the baculovirus DNA. For example, BacVector-1000 DNA and BacVector-2000 DNA (supplied from Novagen) in which the polyhedrin gene has been removed from AcNPV can be used.

**[0083]** The co-transfection for the homologous recombination of the obtained transfer vector and baculovirus DNA into insect cells can be performed using a commercially available vector transfection kit (BacVector Transfection Kits, supplied from Novagen) as described above in accordance with the instructions packaged with the vector transfection kit. Thus, the obtained transfer vector can be co-transfected together with the baculovirus DNA into insect cells such as Sf9 cell to yield a recombinant baculovirus.

**[0084]** In the present disclosure, in accordance with the above method of producing a recombinant baculovirus, a transfer vector that is any one of pCAP-PfCSP, pCAP-PfCSP/272, pCAP-PfCSP/467, pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, pCAP-PfCSP(A361E)/467, pCAP-PfCSP-76, pCAP-PfCSP-76/467, pCAP-PfCSP+209, pCAP-PfCSP+209/467, pCAP-PfCSP+76/209, pCAP-PfCSP+76/209/467, pCAP-HA1/Anhui, pCAP-HA1/Anhui/272, pCAP-HA1/Anhui/467, pCAP-HA1/Vietnam, pCAP-HA1/Vietnam/51, pCAP-HA1/Vietnam/101, pCAP-HA1/Vietnam/154, pCAP-HA1/Vietnam/201, pCAP-HA1/Vietnam/272, pCAP-HA1/Vietnam/467, pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, pCAP-AH/520/467, pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, pCAP-VN/520/467, pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, pCAP-CO/205/467, pCA39-HA1/Anhui, pCA64-HA1/Anhui, pCA39-PfCSP(A361E), pCA64-PfCSP(A361E), pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76-VSV, pCAP-CO/205/VSV, pDual-Pfs25-PfCSP-gp64, and pDual-PfMSP1-PfCSP-gp64, and a baculovirus DNA can be co-transfected into Sf9 insect cells to yield a recombinant baculovirus of any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-

HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

**[0085]** In addition to the above method of producing a recombinant baculovirus, other methods of producing a recombinant baculovirus include, for example, a method of using a transposon as a phagemid (bacmid) having the entire baculovirus genome incorporated therein to efficiently insert a foreign gene into *Escherichia coli*.

**[0086]** According to this method, a bacmid bearing a viral gene is extracted from bacterial cells and transfected into insect cells to thereby easily produce and collect a recombinant baculovirus.

**[0087]** The purification of the recombinant baculovirus of the present disclosure obtained by the above method of producing a recombinant baculovirus can be performed by using known virus purification methods.

**[0088]** For the purification of the recombinant baculovirus, for example, 0.5 to 1.0 mL of a stock virus obtained by the above method of producing a recombinant baculovirus is inoculated into insect cells ( $1 \times 10^7$  cells/10 cm dish), such as Sf9 cells, the culture supernatant is collected several days after the infection, and a virus pellet obtained by centrifugation is suspended in a buffer, such as PBS. The resulting suspension is subjected to a sucrose gradient of 10 to 60%, and then centrifuged (25,000 rpm, 60 minutes, 4°C) to collect a virus band. The collected virus is further suspended in PBS, subsequently centrifuged (under the same conditions as above), and the resulting purified recombinant virus pellet is stored at 4°C in a buffer, such as PBS.

**[0089]** The infectivity titer of the above resulting purified recombinant virus can be measured by plaque assay (BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL, Oxford University Press, 1994) using insect cells such as Sf9 cells.

**[0090]** In the recombinant viruses described in the Examples, the N terminus of the baculovirus protein gp64 is exposed outside the particle, whereas the C terminus thereof is inside the particle. Therefore, if the protein encoded by a foreign gene having the desired immunogenicity is fused to the N terminus of gp64, the entity thereof is exposed as a component of the viral particle outside the viral protein particle in insect cells, and thus the antigen is more easily presented, which is suitable for use as the vaccine formulation of the present invention.

### **(3) Pharmaceutical Composition of the Present Invention (Pharmaceutical Containing the Recombinant Baculovirus of the Present Invention as an Active Ingredient)**

**[0091]** The recombinant baculovirus used in the present invention, which is an active ingredient of the pharmaceutical composition of the present invention, can be obtained by the genetic engineering techniques shown in the above (2).

**[0092]** The pharmaceutical composition of the present invention essentially contains as the active ingredient a recombinant baculovirus obtained by homologous recombination of a baculovirus DNA and a transfer vector constructed so that a fusion gene obtained by the fusion of an immunogenic foreign gene used in the present invention to a gene encoding the amino acid sequence of a protein capable of being a component of the viral particle can be expressed in insect cells and vertebrate cells, particularly cells of mammals, including humans.

**[0093]** The present disclosure particularly provides a pharmaceutical composition comprising as an active ingredient a specific recombinant baculovirus that is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-

CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64. disclosure

**[0094]** The recombinant baculovirus of the present disclosure that is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/5, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64, used as an active ingredient of the pharmaceutical composition of the present disclosure, has the action of enhancing infection-preventing effects against an infectious antigen and reducing the infectivity titer. Utilizing this action and activity, the recombinant baculovirus can be used for the treatment of diseases associated with infection of target cells and tissues. Examples of target cells affected by such infection include blood cells, hepatic cells, renal cells, brain cells, lung cells, epithelial cells, and muscular cells. Examples of tissues comprising such cells include the lung, liver, kidney, brain, arteries and veins, the stomach, intestines, urethra, skin, and muscle.

**[0095]** The pharmaceutical composition enhances infection-preventing effects against infectious antigens, for example, malaria antigens such as sporozoite surface antigens (CSP and TRAP) of malaria parasites, merozoite surface membrane protein MSP1, malaria S antigen secreted from erythrocytes infected with malaria, PfEMP1 protein present in the knobs of erythrocytes infected with malaria, SERA protein, TRAMP protein, AMA1 protein, and Pfs25 known as a transmission-blocking antigen; and influenza antigens such as HA antigen, NA antigen, M2 antigen, and NP antigen, and reduces the infectivity titer (e.g., viral infectivity titer), thereby increasing the survival period and survival rate of mammals including humans, compared to the group not administered the pharmaceutical composition of the present disclosure. Therefore, the pharmaceutical composition is particularly useful as a preventive or therapeutic agent for malaria and influenza virus infections.

**[0096]** The pharmaceutical composition of the invention has the action of enhancing infection-preventing effects against infectious antigens and reducing the infectivity titers, and is therefore useful as a preventive or therapeutic agent for infectious diseases caused by pathogens such as malaria, and complications thereof.

**[0097]** By using a gene immunogenic to non-human vertebrates as an immunogenic foreign gene of the transfer vector to obtain a recombinant baculovirus used as an active ingredient of the pharmaceutical composition of the present disclosure, for example, a chicken influenza vaccine can be prepared, which has the action of enhancing infection-preventing effects against the infectious antigen and reducing the infectivity titer. By utilizing this action and activity, the pharmaceutical composition can be used for the treatment of diseases associated with the infection of target cells and tissues.

**[0098]** The pharmaceutical composition of the present invention can be prepared as a composition containing a pharmaceutically effective amount of the recombinant baculovirus used in the invention and a pharmaceutically acceptable carrier.

**[0099]** The infection-preventing effect of the recombinant baculovirus used in the present invention in vertebrates, particularly mammals, including humans, or mammalian cells can be provided, for example, by administering the pharmaceutical composition containing the recombinant baculovirus used in the present invention and additives for pharmaceutical administration to vertebrates, particularly mammals, including humans, by the intramuscular, subcutaneous, intracutaneous, intraperitoneal, nasal, or respiratory route, and then immunizing the vertebrates with the pharmaceutical composition containing the recombinant baculovirus used in the present invention as an active ingredient several times. Respiratory administration of the pharmaceutical composition of the invention is particularly preferable.

**[0100]** The infection-preventing effect can be evaluated by comparing the survival rate of vertebrates, particularly mammals, including humans, that have been immunized with the pharmaceutical composition of the invention several times and then infected with a target pathogen with the survival rate of those not administered with the pharmaceutical composition.

**(4) Vaccine of the present invention**

**[0101]** The recombinant baculovirus that is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/159, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64 used as the active ingredient of the pharmaceutical composition of the present disclosure can enhance pathogen infection-preventing effects as described in the Examples below, and an expressed fusion product of the DNA sequence obtained by fusion of a gene encoding the amino acid sequence of a protein capable of being a component of viral particles and a foreign gene with the desired immunogenicity of the present disclosure capable of reducing the infectivity titer is produced as viral particles budded from insect cells. When the pharmaceutical composition of the invention is administered in the form of viral particles to vertebrates, particularly mammals, including humans, a foreign antigen protein contained as a component of viral particles promotes acquired immunity (humoral immunity and cellular immunity), and further, the antigenic protein as an expressed product of the fusion DNA sequence seems to promote acquired immunity (humoral immunity and cellular immunity) in vertebrate cells, particularly cells of mammals, including humans. Thus, the recombinant baculovirus used in the present invention is useful as a vaccine.

**[0102]** The present disclosure particularly provides a vaccine containing as an active ingredient a specific recombinant baculovirus that is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA2/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

**[0103]** As in the pharmaceutical composition of the above (3), the vaccine enhances infection-preventing effects against infectious antigens such as malaria antigens such as sporozoite surface antigens (CSP and TRAP) of the malaria parasite, merozoite surface membrane protein MSP1, malaria S antigen secreted from erythrocytes infected with malaria, PfEMP1 protein present in the knobs of erythrocytes infected with malaria, SERA protein, TRAMP protein, and AMA1 protein; and influenza antigens such as influenza virus HA antigen, influenza virus NA antigen, influenza virus M2 antigen, and influenza virus NP antigen; the vaccine also reduces the infectivity titer (e.g., the viral infectivity titer), thereby increasing the survival period and survival rate of mammals, including humans, compared with the group not administered with the pharmaceutical composition of the present disclosure. Thus, the vaccine is particularly useful as a preventive or therapeutic agent for malaria and influenza virus infection.

**[0104]** The vaccine of the disclosure enhances infection-preventing effects against infectious antigens and reduces the infectivity titer, and is therefore useful as a preventive or therapeutic agent for infectious diseases caused by pathogens such as influenza viruses and malaria, and complications thereof.

**[0105]** By using a gene immunogenic to non-human vertebrates as an immunogenic foreign gene of the transfer vector to obtain a recombinant baculovirus used as an active ingredient of the pharmaceutical composition of the present disclosure, for example,

a chicken influenza vaccine, that can enhance infection-prevention effects against the infectious antigen and reduce the infectivity titer, can be prepared. By utilizing this activity, the vaccine can be used for the treatment of diseases associated with the infection of target cells and tissues.

**[0106]** The recombinant baculovirus that is any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/15, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64, used as an active ingredient of the vaccine of the present disclosure, can enhance infection-preventing effects against an infectious antigen and reduce the infectivity titer.

**[0107]** By utilizing this activity, the recombinant baculovirus can be used for the treatment of diseases associated with the infection of target cells and tissues.

**[0108]** Examples of target cells affected by such infection include blood cells, hepatic cells, renal cells, brain cells, lung cells, epithelial cells, and muscular cells. Examples of tissues containing such cells include lung, liver, kidney, brain, arterial and venous veins, stomach, intestine, urethra, skin, and muscle tissues.

**[0109]** The vaccine of the present disclosure can be prepared as a pharmaceutical composition of the above (3) containing a pharmaceutically effective amount of the recombinant baculovirus of the disclosure (any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64), and a pharmaceutically acceptable carrier.

**[0110]** The vaccine can be prepared in the form of a pharmaceutical composition by using a pharmaceutically acceptable carrier as used in the pharmaceutical composition of the above (3), according to a standard method. Examples of carriers include physiologically acceptable solutions, such as sterile saline and sterile buffered saline.

**[0111]** The vaccine (the formulation is hereinafter the same as in the pharmaceutical composition) can be prepared as a liposomal formulation containing as an active ingredient the recombinant baculovirus of the disclosure (any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui,

AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64), and can be used in combination with an adjuvant.

**[0112]** Specific examples of the vaccine (the pharmaceutical composition) of the present invention include liposomal formulations. The liposomal formulation may be one in which the recombinant baculovirus used in the present invention is retained in a liposome containing an acidic phospholipid as a membrane component, or containing a neutral phospholipid and an acidic phospholipid as membrane components.

**[0113]** The acidic phospholipid and neutral phospholipid used as membrane components are not particularly limited, and various lipids commonly used for liposomal formulations can be used alone or in a combination of two or more.

**[0114]** The liposome membrane is produced in accordance with a standard method by using an acidic phospholipid alone or together with a neutral phospholipid. When an acidic phospholipid is used together with a neutral phospholipid, the proportion of the acidic phospholipids in the liposome membrane components is preferably about 0.1 to about 100 mol%, more preferably 1 to 90 mol%, and even more preferably about 10 to about 50 mol%.

**[0115]** To prepare the liposome, for example, cholesterol or the like can be added. This can control the fluidity of phospholipids and facilitates the liposome preparation. In general, the cholesterol is preferably added in an equivalent amount or less, and preferably in a 0.5-fold amount to an equivalent amount by weight, to the phospholipid.

**[0116]** The mixing ratio of the acidic phospholipid to the active ingredient in the liposomal formulation is about 0.5 to about 100 equivalents, preferably about 1 to about 60 equivalents, and more preferably about 1.5 to about 20 equivalents.

**[0117]** The recombinant baculovirus used in the present invention as an active ingredient is used in an amount of several mol% to several tens mol%, preferably about 5 to about 10 mol%, and usually around 5 mol%, based on the total amount of the lipids.

**[0118]** The production, concentration, and particle diameter control of the liposomal formulation can be performed in accordance with standard methods. If desired, the various additives as described above can also be incorporated into the liposomal formulation.

**[0119]** To produce the liposomal formulation, the recombinant baculovirus used in the invention as an active ingredient can be used in the form of being bound to a fatty acid (e.g., behenic acid, stearic acid, palmitic acid, myristic acid, and oleic acid), an alkyl group, a cholesteryl group, or the like. The liposomal formulation containing such bound components can also be produced in accordance with a standard method (see, for example, *Long Circulating Liposomes: Old Drugs, New Therapeutics.*, M. C. Woodle, G. Storm, Eds: Springer-Verlag Berlin (1998)).

**[0120]** The vaccine (pharmaceutical composition) of the present invention can preferably be used as a vaccine composition. The vaccine is preferably used in combination with a pharmaceutically effective amount of an adjuvant to enhance the anti-infection (anti-malaria) effects.

**[0121]** Any adjuvant commonly used for this type of vaccine can be used as the adjuvant. Examples of such adjuvants include Freund's complete adjuvant, muramyl dipeptide, aluminium hydroxide, BCG, IL-12, N-acetylmuramine-L-alanyl-D-isoglutamine (MDP), thymosin  $\alpha$ 1, and QS-21. The amount of adjuvant used can be suitably selected according to the degree of symptoms, such as softening of the skin, pain, erythema, fever, headache, and muscular pain, which might be expressed as part of the immune response in humans or animals after the administration of this type of vaccine.

**[0122]** The vaccine (pharmaceutical composition) of the present invention may be used in combination with other publicly known pharmaceutical products, such as immune response-promoting peptides and antibacterial agents (synthetic antibacterial agents).

**[0123]** The vaccine (pharmaceutical composition) may further contain other drugs and additives. Examples thereof include drugs that aid intracellular uptake of the recombinant baculovirus used in the present invention, such as calcium ions. The liposome, and other drugs and additives that facilitate the transfection, such as fluorocarbon emulsifiers, cochleates, tubules, golden particles, biodegradable microspheres, and cationic polymers, can also be used.

**[0124]** The amount of the active ingredient contained in the vaccine (pharmaceutical composition) (drug) of the present invention is not particularly limited and can be selected from a wide range as long as it is a pharmaceutically effective amount. The dosage

of the vaccine (pharmaceutical composition) is not particularly limited, and can be appropriately selected from a wide range according to the desired therapeutic effect, the administration method (administration route), the therapeutic period, the patient's age, gender, and other conditions, etc.

**[0125]** When the recombinant baculovirus as an active ingredient of the vaccine (pharmaceutical composition) of the present invention is administered to a human, the recombinant baculovirus is administered in an amount corresponding to  $10^2$  to  $10^{14}$  PFU, preferably  $10^5$  to  $10^{12}$  PFU, and more preferably  $10^6$  to  $10^{10}$  PFU per patient, calculated as the PFU of the recombinant virus.

**[0126]** The dosage of the recombinant baculovirus (any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64), used as an active ingredient of the vaccine (pharmaceutical composition) of the present disclosure, can be selected from a very wide range, in terms of the amount of expressible DNA introduced into the vaccine host or the amount of transcribed RNA. These amounts also depend on the strength of the transcription and translation promoters used in the transfer vector of the disclosure.

**[0127]** The vaccine (pharmaceutical composition) of the present invention is administered by directly injecting a recombinant baculovirus suspension prepared by suspending the recombinant baculovirus in PBS (phosphate buffered saline) or saline into a local site (e.g., into the lung tissue, liver, muscle or brain), or by nasal or respiratory inhalation, or by intravascular (e.g., intra-arterial, intravenous, and portal venous), subcutaneous, intracutaneous, or intraperitoneal administration. The vaccine of the invention is preferably administered by respiratory inhalation.

**[0128]** The vaccine (pharmaceutical composition) of the present invention is preferably administered more than once. More specifically, after the initial administration, additional vaccinations are preferably performed once to several times while the condition is observed. This can enhance the desired effect. After the administration of the vaccine (pharmaceutical composition), booster immunization with a pharmaceutical composition containing the recombinant baculovirus of the present disclosure (any one of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64) can be performed. Further, use of various other drugs with the vaccine of the invention, as mentioned above, may enhance the therapeutic effect achieved by the administration of the vaccine (pharmaceutical composition).

**[0129]** In one embodiment of the vaccine (pharmaceutical composition) of the present invention, the recombinant baculovirus, used as one of the active ingredients of the vaccine (pharmaceutical composition) of the invention, is generally prepared by homologous recombination of a baculovirus DNA and a transfer vector into which a fusion gene obtained by fusion of a foreign gene having the desired immunogenicity and a gene encoding a protein capable of being a component of viral particles has been introduced. The recombinant baculovirus is mixed, in an injectable dosage form (a solution, suspension or emulsion), with a pharmaceutically acceptable carrier (i.e., non-toxic to humans and other vertebrates in the dosage and concentration used, and

compatible with other ingredients in the formulation) to produce a formulation. Preferably, the formulation thus obtained does not contain oxidizing agents or any other compound that is publicly known to be harmful to recombinant baculovirus.

**[0130]** The carrier may contain a trace amount of additives, such as substances that enhance the isotonicity and chemical stability. Such additives are non-toxic to mammals, including humans, in the dosage and concentration used, and examples thereof include buffers such as phosphoric acid, citric acid, succinic acid, acetic acid, and other organic acids, and salts thereof; antioxidants such as ascorbic acid; low molecular weight (e.g., less than about 10 residues) polypeptides (e.g., polyarginine and tripeptide) proteins (e.g., serum albumin, gelatin, and immunoglobulin); amino acids (e.g., glycine, glutamic acid, aspartic acid, and arginine); monosaccharides, disaccharides, and other carbohydrates (e.g., cellulose and derivatives thereof, glucose, mannose, and dextrin), chelating agents (e.g., EDTA); sugar alcohols (e.g., mannitol and sorbitol); counterions (e.g., sodium); nonionic surfactants (e.g., polysorbate and poloxamer); and PEG.

**[0131]** The pharmaceutical vaccine (composition) containing the recombinant baculovirus can typically be stored as an aqueous solution or a lyophilized product in a unit or multiple dose container such as a sealed ampoule or a vial.

**[0132]** The present disclosure further provides a method of preventing malaria or influenza infection, or a method of treating malaria or influenza, comprising administering an effective amount of the recombinant baculovirus, vaccine, formulation, and pharmaceutical composition of the disclosure to a subject. The present disclosure further provides a method of immunostimulation comprising administering an effective amount of the recombinant baculovirus, vaccine, formulation, and pharmaceutical composition of the disclosure to a subject. Examples of the subjects used include those that may be infected with malaria parasites or influenza viruses, such as humans and other animals (such as mammals, birds, reptiles, fish, and amphibians), and those infected with malaria parasites or influenza viruses. The influenza virus with which the subject is infected is preferably an influenza A virus, and more preferably an influenza A subtype H1 virus, or an influenza A subtype H3 virus. Examples of malaria parasites include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*.

**[0133]** The recombinant baculovirus of the present disclosure is formed alone or together with a pharmaceutically acceptable carrier into a vaccine, formulation, or pharmaceutical composition, and administered to the subject.

**[0134]** The administration route may be, for example, any administration route mentioned above. The pharmaceutically acceptable carrier for use in the present invention can be suitably selected from carriers commonly used in this technical field, according to the form of the pharmaceutical composition to be produced.

**[0135]** For example, when the pharmacological composition is formed into an aqueous solution, purified water (sterile water) or a physiological buffer solution can be used as the carrier. When the pharmaceutical composition is formed into other appropriate solutions, organic esters capable of being injected, such as glycol, glycerol and olive oil, can be used as the carrier. The composition may contain stabilizers, excipients and the like commonly used in this technical field, and particularly in the field of vaccine formulations.

**[0136]** The amount of recombinant baculovirus in the vaccine, formulation, or pharmaceutical composition of the present invention is not particularly limited, and can be suitably selected from a wide range. In general, the amount of recombinant baculovirus in the composition is preferably about 0.0002 to about 0.2 (w/v %), and more preferably 0.001 to 0.1 (w/v %). The administration method of the recombinant baculovirus, vaccine, formulation, or pharmaceutical composition of the invention is not particularly limited, and can be suitably selected according to the dosage form, the patient's age, gender and other conditions, the severity of the disease, etc. A preferable dosage form thereof is a form for parenteral administration, such as injections, drops, nasal drops, and inhalants. When the composition is formed into an injection or drops, the injection can be intravenously administered as mixed with a replacement fluid such as a glucose solution or an amino acid solution as required, or can be administered intramuscularly, intracutaneously, subcutaneously or intraperitoneally.

**[0137]** The daily dosage of the recombinant baculovirus, vaccine, formulation, or pharmaceutical composition of the present invention may vary depending on the subject's condition, body weight, age, gender, etc., and therefore cannot be completely specified. However, the dosage is usually such that the recombinant baculovirus is administered in an amount of 0.001 to 100 mg per kg of body weight per day. The vaccine, formulation, or composition of the invention can be administered in one or more administrations per day.

**[0138]** When the recombinant baculovirus as an active ingredient of the vaccine (formulation or pharmaceutical composition) of the present invention is administered, the recombinant baculovirus is administered in an amount corresponding to  $10^2$  to  $10^{14}$  PFU, preferably  $10^5$  to  $10^{12}$  PFU, and more preferably  $10^6$  to  $10^{10}$  PFU per patient, calculated as the PFU of the recombinant

virus.

[0139] The vaccine (composition) of the present invention is administered according to Good Medical Practice, considering the clinical condition (for example, the condition to be prevented or treated) of each patient, the delivery site of the vaccine (composition) containing the recombinant baculovirus, the target tissue, the administration method, the dosage regimen, and other factors publicly known to those skilled in the art. Therefore, the proper dosage of the vaccine (composition) herein is determined in consideration of the above.

## EXAMPLES

[0140] The present invention will be described below in more detail with reference to Examples. These Examples are merely examples of the present invention, and do not limit the present invention.

### Example 1: Transfer Vector Plasmid and Method for Production thereof of the Present Invention

#### (1) Construction of Transfer Vector Plasmids pCAP-PfCSP, pCAP-PfCSP/272, and pCAP-PfCSP/467

##### (1.1) Construction of Plasmid pBACsurf-Hsp65

[0141] An Hsp65 gene was obtained by extracting genomic DNA from *M. tuberculosis* H37Rv strain using a QIAamp DNA Midi Kit (Qiagen), and cloning by PCR. More specifically, the genomic DNA extracted from the *M. tuberculosis* H37Rv strain was amplified by PCR using primers phsp65-F1 (5'-AATAATAGATCTAATGGCCAAGACAATTGCGTACGACGAAGA -3' (SEQ ID NO: 1); the BglII site is underlined) and phsp65-R1 (5'-AATCCAATGCGGCCGCGGGAATTCGATTCTCTGCAGGTCAGAAATCCATGCCACCCATGTCGCC -3' (SEQ ID NO: 2); the NotI site is underlined). The PCR product was purified, cleaved with restriction enzymes BglII and NotI, ligated to pcDNA3.1(+) (from Invitrogen) digested with BamHI and NotI. The resulting plasmid was designated pcDNA-hsp65. PCR was performed with pcDNA-hsp65 as a template using primers phsp65-F2 (5' - CACCCTGCAGGACTACAAGGACGACGATGACAAGGAATTCATGGCCAAGAC AATTGCGTACGACGAAGAGGCC - 3' (SEQ ID NO: 3); the Sse8387I and EcoRI sites are underlined, and the FLAG sequence is italicized), and phsp65-R2 (5' - CCCGGGCGAAATCCATGCCACCCATGTCGCCGCCACC - 3' (SEQ ID NO: 4); the Cfr9I site is underlined). The resulting Hsp65 gene DNA fragment was cloned into pENTR/D-TOPO (from Invitrogen), then cleaved with Sse8387I/Cfr9I and inserted into the PstI/Cfr9I site of pBACsurf-CSP (Yoshida et al., Virology 316: 161-70, 2003). The plasmid thus constructed was designed pBACsurf-Hsp65.

##### (1.2) Construction of Plasmid pENTR-gp64

[0142] PCR was performed with pBACsurf-1 (from Novagen) as a template using primers pPolh-F2 (5' - CACCCGGACCGGATAATTAATGATAACCATCTCGCAAATAAATAAG - 3' (SEQ ID NO: 5); the RsrII site is underlined), and pgp64-R2 (5' - GGTACCATATTGTCTATTACGGTTTCTAATCATAAC - 3' (SEQ ID NO: 6); the KpnI site is underlined). The resulting gp64 gene DNA fragment was inserted into pENTR/D-TOPO to construct a plasmid pENTR-gp64. The plasmid thus constructed was designated pENTR-gp64.

##### (1.3) Construction of Transfer Vector pDual-Hsp65-gp64

[0143] pBACsurf-Hsp65 was cleaved with PstI/Cfr9I, and the hsp65 gene DNA fragment was inserted into the PstI/Cfr9I site of pENTR-gp64 to construct a plasmid pENTR-Hsp65-gp64. The pENTR-Hsp65-gp64 was cleaved with RsrII/KpnI, and a DNA fragment consisting of a polyhedrin promoter and hsp65-gp64 gene was inserted into pTriEx-3.1 (Novagen) cleaved with RsrII and KpnI to construct a transfer vector plasmid pDual-Hsp65-gp64 enabling the expression of a fusion protein of Hsp65 antigen and gp64 protein in mammalian and insect cells under the control of the desired dual promoter consisting of CMA promoter and polyhedrin promoter.

**(1.4) Construction of Transfer Vector pDual-H1N1/HA1-gp64**

[0144] RNA was extracted from a culture supernatant of MDCK cells infected with influenza virus PR/8/34 strain using a QIAamp MinElute Virus Spin Kit (from Qiagen), and amplified by RT-PCR using primers HA-f (5' - CCTGCAGGTATGAAGGCAAACCTACTGGTC - 3' (SEQ ID NO: 7) ; the SbfI site is underlined) and HA-r (5' - GCCCGGGCGATGCATATTCTGCA - 3' (SEQ ID NO: 8); the SbfI site is underlined). The resulting influenza virus HA gene fragment was cloned into pCR-Blunt II-TOPO (from Invitrogen). The resulting plasmid was designated as pCR-Blunt-HA. PCR was performed with the pCR-Blunt-HA as a template using primers pHA-F1 (5' - CACCGAATTCGACACAATATGTATAGGCTACCATGCG-3' (SEQ ID NO: 9); the EcoRI site is underlined) and pHA-R1 (5' - CCCGGGCACCTCTGGATTGGATGGACGGAATG - 3' (SEQ ID NO: 10); the Cfr9I site is underlined). The resulting H1N1/HA1 gene DNA fragment was cloned into pENTR/D-TOPO, then cleaved with EcoRI/Cfr9I and inserted into the EcoRI/Cfr9I site of pDual-Hsp65-gp64 to construct a plasmid pDual-H1N1/HA1-gp64.

**(1.5) Construction of Plasmid pBACsurf-HA1**

[0145] pDual-H1N1/HA1-gp64 was cleaved with EcoRI/CfrI, and the DNA fragment of H1N1/HA1 gene was inserted into pBACsurf-Hsp65 digested with EcoRI and CfrI to construct a plasmid pBACsurf-HA1.

**(1.6) Construction of Plasmid pCP-H1N1/HA1-gp64**

[0146] PCR was performed with pBACsurf-HA1 as a template using Polh-f RsrII (5' - GGGCGGACCGGATAATTAATGATAACCATCTCG - 3' (SEQ ID NO: 11); the RsrII site is underlined) and GP64-r DralIII (5' - GGGCACTTAGTGATATTGTCTATTACGGTTTCTAATC - 3' (SEQ ID NO: 12); the DralIII site is underlined). The resulting DNA fragment was inserted into pDual-H1N1/HA1-gp64 digested with RsrII and DralIII to yield pCP-H1N1/HA1-gp64.

**(1.7) Construction of Plasmid pCAP-H1N1/HA1-gp64**

[0147] pCP-H1N1/HA1-gp64 was cleaved with restriction enzymes RsrII and DralIII to prepare HA1 and gp64 gene fragments. The fragments were inserted into a vector prepared by digesting pTriEx-1.1 (from Novagen) with restriction enzymes RsrII and DralIII to yield a transfer vector plasmid pCAP-H1N1/HA1-gp64 enabling the expression of a fusion protein of HA1 antigen and gp64 protein in mammalian and insect cells under the control of the desired dual promoter consisting of CAG promoter and polyhedrin promoter.

**(1.8) Construction of Plasmid pCAP-H1N1/NP-gp64**

[0148] RT-PCR was performed with genomic RNA of influenza virus PR/8/34 strain as a template using NP-f EcoRI (5'- ACGGAATTCATTCAATTCAAACCTGGA - 3' (SEQ ID NO: 13); the EcoRI site is underlined) and NP-r Cfr9I (5'- GATCCCGGGCCTTGTCAATGCTGAATGGCAA - 3' (SEQ ID NO: 14); the Cfr9I site is underlined). The obtained fragments were digested with restriction enzymes EcoRI and Cfr9I, and inserted into pCAP-H1N1/HA1-gp64 digested with restriction enzymes EcoRI and Cfr9I to yield pCAP-H1N1/NP-gp64.

**(1.9) Construction of Plasmids pCAP-H1N1/NP/272 and pCAP-H1N1/NP/467**

[0149] PCR was performed using gp64(272)-f (5' - GACTCCCCGGGTCGAGCACCGAGTCAAGAAG - 3' (SEQ ID NO: 15); the XmaI site is underlined), gp64(467)-f (5'-GACTCCCCGGGACATCACTTCCATGGCTGAA-3' (SEQ ID NO: 16); the XmaI site is underlined), and GP64-r DralIII (5'-GGGCACTTAGTGATATTGTCTATTACGGTTTCTAATC-3' (SEQ ID NO: 12); the DralIII site is underlined) of pCAP-H1N1/HA1-gp64. The obtained fragments were digested with restriction enzymes XmaI and DralIII, and inserted into pCAP-H1N1/NP-gp64 digested with XmaI and DralIII to yield pCAP-H1N1/NP/272 and pCAP-H1N1/NP/467.

**(1.10) Construct of Transfer Vectors pCAP-PfCSP, cCAP-PfCSP/272 and pCAP-PfCSP/467**

[0150] *P. falciparum* genomic DNA was extracted from human erythrocytes infected with *Plasmodium falciparum* 3D7 strain using a QIAamp DNA Midi Kit (from Qiagen). A PfCSP gene was cloned by PCR with this genomic DNA as a template according to the following method. The PCR was performed using primers PfCSP-f(19)(5' - GACTCTGCAGTTATTCCAGGAATACCAGTGCTATGGAAG - 3' (SEQ ID NO: 17); the PstI site is underlined) and PfCSP-r(373) (5'-CGATCCCGGGCTTTTCCATTTACAAATTTTTTTTCAATATC - 3' (SEQ ID NO: 18); the XmaI site is underlined). The resulting DNA fragment was inserted into pCAP-H1N1/NP-gp64, pCAP-H1N1/NP/272, and pCAP-H1N1/NP/467, each digested with PstI and XmaI. The constructed plasmids were designated pCAP-PfCSP, pCAP-PfCSP/272, and pCAP-PfCSP/467.

[0151] The GenBank accession number of the amino acid sequence of the *Plasmodium falciparum* 3D7 circumsporozoite (CS) protein is XP\_001351122.

**(2) Construction of Transfer Vector pDual-Pfs25-PfCSP-gp64****(2.1) Construction of Plasmid pDual-PbAMA1D123-gp64**

[0152] A blood sample was collected from a BALB/c mouse infected with malaria parasite *P. berghei* ANKA strain, and *P. berghei* genomic DNA was extracted using a QIAamp DNA Midi Kit (Qiagen). A PbAMA1 gene domain 123 (D123) was cloned by PCR with this genomic DNA as a template according to the following method. PCR was performed using primers pAMA-F1 (5' - CACCGAATTCAATCCATGGGAAAAGTATACGGAAAAATAT - 3' (SEQ ID NO: 19); the EcoRI site is underlined) and pAMA-R1 (5' - CCCGGGCTTCTCTGGTTTGATGGGCTTTCATATGCAC - 3' (SEQ ID NO: 20); the Cfr9I site is underlined). The resulting PbAMA1D123 DNA fragment was cloned into pENTR/D-TOPO, then cleaved with EcoRI/Cfr9I and inserted into pBACsurf-Hsp65 digested with EcoRI and Cfr9I. The constructed plasmid was designated pBACsurf-PbAMA1D123.

[0153] Subsequently, the pBACsurf-PbAMA1D123 was cleaved with EcoRI/Cfr9I, and the PbAMA1D123 gene DNA fragment was inserted into pDual-Hsp65-gp64 digested with EcoRI and Cfr9I to yield a plasmid pDual-PbAMA1D123-gp64.

**(2.2) Construction of Plasmid pDual-PfCSP-gp64**

[0154] A PfCSP gene was cloned by PCR with *P. falciparum* genomic DNA as a template according to the following method. The PCR was performed using primers pPfCSP-F1 (5' - CACCGAATTCTTATTCCAGGAATACCAGTGCTATGGAAGT-3' (SEQ ID NO: 21); the EcoRI site is underlined) and pPfCSP-R1 (5'-CCCGGGCTTTTCCATTTTACAAATTTTTTTTC-3' (SEQ ID NO: 22); the Cfr9I site is underlined). The resulting PfCSP DNA fragment was cloned into pENTR/D-TOPO, then cleaved with EcoRI/Cfr9I and inserted into pDual-PbAMA1D123-gp64 digested with EcoRI and Cfr9I. The constructed plasmid was designated pDual-PfCSP-gp64.

**(2.3) Construction of Transfer Vector pDual-Pfs25-PfCSP-gp64**

[0155] A Pfs25 gene was cloned by PCR with *P. falciparum* genomic DNA as a template according to the following method. The PCR was performed using primers pPfs25-F1 (5' - CACCGAATTCAAAGTTACCGTGGATACTGTATGCAAAAGAGGA - 3' (SEQ ID NO: 23); the EcoRI site is underlined) and pPfs25-R2 (5' - CAATTGAGATCCGCCGCCACCGCCACAGTACATATAGAGCTTTCATTATCTATTATAAATCCAT C - 3' (SEQ ID NO: 24); the MunI site is underlined). The resulting Pfs25 DNA fragment was cloned into pENTR/D-TOPO, then cleaved with EcoRI/MunI, and inserted into pDual-PfCSP-gp64 digested with EcoRI. The constructed plasmid was designated pDual-Pfs25-PfCSP-gp64.

**(3) Construction of Transfer Plasmid pDual-PfMSP1-PfCSP-gp64**

[0156] A PfMSP1 gene was cloned by PCR with *P. falciparum* genomic DNA as a template according to the following method. The

PCR was performed using primers pPfMSPH9-F1 (5' - CACCGAATTCAACATTTTACAACACCAATGCGTAAAAAAC - 3'; (SEQ ID NO: 25); the EcoRI site is underlined) and pPfMSP119-R2 (5' - CAATTGAGATCCGCCGCCACCGCCACCGTTAGAGGAAGTGCAGAAAATACCATCGAAAAGTGGG - 3' (SEQ ID NO: 26); the MunI site is underlined). The resulting PfMSP119 DNA fragment was cloned into pENTR/D-TOPO, then cleaved with EcoRI and MunI, and inserted into pDual-PbCSP-gp64 digested with EcoRI. The constructed plasmid was designated pDual-PfMSP1-PfCSP-gp64.

#### **(4) Construction of the Transfer Vector Plasmids pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, and pCAP-PfCSP(A361E)/467**

[0157] PCR was performed with pCAP-PfCSP using PfCSP-f(19)(5'-GACTCTGCAGTTATTCCAGGAATACCAGTGCTATGGAAG-3'; (SEQ ID NO: 17); the PstI site is underlined) and PfCSP-r(373 A361E) (5'-CGATCCCGGGCTTTTTCCATTTTACAAATTTTTTTTTCAATATCATTTTC-3'; (SEQ ID NO: 27); the XmaI site is underlined). The obtained DNA fragment was cleaved with PstI and XmaI, and inserted into pCAP-H1N0/NP-gp64, pCAP-H1N1/NP/272, and pCAP-H1N1/NP/467, each digested with PstI and XmaI. The constructed plasmids were designated pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, and pCAP-PfCSP(A361E)/467.

#### **(5) Construction of Transfer Plasmids pCAP-PfCSP-76 and pCAP-PfCSP-76/467**

[0158] PCR was performed with pCAP-PfCSP(A361E) using PfCSP-f(76) (5' - GACTCTGCAGGATGATGGAAATAACGAAGACAACG - 3'; (SEQ ID NO: 28); the PstI site is underlined) and PfCSP-r(373 A361E) (5' - CGATCCCGGGCTTTTTCCATTTTACAAATTTTTTTTTCAATATCATTTTC - 3'; (SEQ ID NO: 27); the XmaI site is underlined). The resulting DNA fragment was cleaved with PstI and XmaI, and then inserted into pCAP-H1N1/NP-gp64 and pCAP-H1N1/NP/467, each cleaved with PstI and XmaI. The constructed plasmids were designated pCAP-PfCSP-76 and pCAP-PfCSP-76/467.

#### **(6) Construction of Transfer Plasmids pCAP-PfCSP+209 and pCAP-PfCSP+209/467**

[0159] An artificial gene sequence (PfCSP+: SEQ ID NO: 29) was prepared from the amino acid sequence of PfCSP of *P. falciparum* 3D7 strain (in which, however, the A at the 361-position was replaced by E) using codons frequently used in Sf9 and human cells. Using the obtained artificial gene sequence as a template, PCR was performed using PfCSP-f(+209) (5'-GACTCTGCAGAACGCTAATCCAACGCTAATCCAACGCTAATCCCAATGCC -3' (SEQ ID NO: 30); the PstI site is underlined) and PfCSP-r(+A361E) (5'-CGATCCCGGGCTTTTTCCATTTTGCAAATTTTTTT -3' (SEQ ID NO: 31); the XmaI site is underlined). The resulting DNA fragments were cleaved with PstI and XmaI, and then inserted into pCAP-H1N1/NP-gp64 and pCAP-H1N1/NP/467 digested with PstI and XmaI. The constructed plasmids were designated pCAP-PfCSP+209 and pCAP-PfCSP+209/467.

#### **(7) Construction of Transfer Plasmids pCAP-PfCSP+76/209 and pCAP-PfCSP+76/209/467**

[0160] Using the artificial gene sequence (PfCSP+: SEQ ID NO: 29) as a template, PCR was performed using PfCSP-f(+76) (5'-GACTCTGCAGGACGACGGCAACAACGAAGACAACG -3' (SEQ ID NO: 32); the PstI site is underlined), PfCSP-r(+128) (5'-CGTTAGGATCCACATTTGGGTTGGCATTGGG -3' (SEQ ID NO: 33); the BamHI site is underlined), PfCSP-f(+209) BamHI (5'-GACTGGATCCTAACGCTAATCCAACGCTAATCCC -3'; (SEQ ID NO: 34); the BamHI site is underlined), and PfCSP-r(+A361E) (5'-CGATCCCGGGCTTTTTCCATTTTGCAAATTTTTTT -3' (SEQ ID NO: 31); the XmaI site is underlined) from the obtained artificial gene sequence. The resulting DNA fragments were cleaved with PstI/BamHI and BamHI/XmaI, respectively, and then inserted into pCAP-H1N1/NP-gp64 and pCAP-H1N1/NP/467, each digested with PstI and XmaI. The constructed plasmids were designated pCAP-PfCSP+76/209 and pCAP-PfCSP+76/209/467.

#### **(8) Construction of Transfer Plasmids pCAP-HA1/Anhui, pCAP-HA1/Anhui/272, and pCAP-HA1/Anhui/467**

[0161] An artificial gene sequence (SEQ ID NO: 35) was prepared from the amino acid sequence of the hemagglutinin HA1

region of influenza virus H5N1/Anhui/1/05 using codons frequently used in Sf9 and human cells. Using the obtained artificial gene sequence as a template, PCR was performed using AH-F1 (5'-CAGTCTGCAGGACCAGATTTGCATC-3': (SEQ ID NO: 36); the PstI site is underlined) and AH-R4 (5'-CAGTCCCGGGCTCTCTTGCGCCTGC-3': (SEQ ID NO: 37); the XmaI site is underlined). The obtained DNA fragment was cleaved with PstI and XmaI, and then inserted into pCAP-H1N1/NP-gp64, pCAP-H1N1/NP/272 and pCAP-H1N1/NP/467, each digested with PstI and XmaI. The constructed plasmids were designated pCAP-HA1/Anhui, pCAP-HA1/Anhui/272, and pCAP-HA1/Anhui/467.

**[0162]** The GenBank accession number of the amino acid sequence of the hemagglutinin of influenza virus A/H5N1/Anhui/1/05 is ABD28180.

**(9) Construction of Transfer Vector Plasmids pCAP-HA1/Vietnam, pCAP-HA1/Vietnam/51, pCAP-HA1/Vietnam/101, pCAP-HA1/Vietnam/154, pCAP-HA1/Vietnam/201, pCAP-HA1/Vietnam/272, and pCAP-HA1/Vietnam /467**

**[0163]** An artificial gene sequence (SEQ ID NO: 38) was prepared from the amino acid sequence of the HA1 region of the hemagglutinin of influenza virus H5N1/Vietnam/1203/4 using codons frequently used in Sf9 and human cells. Using the obtained artificial gene sequence as a template, PCR was performed using VN-F1 (5'-CAGTCTGCAGGACCAGATCTGTATC-3': (SEQ ID NO: 39); the PstI site is underlined), and VN-R4 (5'-CAGTCCCGGGCTCTCTTCTCCTGC-3': (SEQ ID NO: 40); the XmaI site is underlined). The obtained DNA fragment was cleaved with PstI and XmaI, and inserted into pCAP-H1N1/NP-gp64, pCAP-H1N1/NP/272 and pCAP-H1N1/NP/467, each digested with PstI and XmaI. The constructed plasmids were designated pCAP-HA1/Vietnam, pCAP-HA1/Vietnam/272, and pCAP-HA1/Vietnam/467.

**[0164]** Further, using pCAP-HA1/Vietnam as a template, PCR was performed using gp64(51)-f (5'-GACTCCCGGGTGGAATCACCATCGTGGAGACG-3': (SEQ ID NO: 41); the XmaI site is underlined), or gp64(101)-f (5'-GACTCCCGGGATTGCTTATGTGGAGCATCAGG-3': (SEQ ID NO: 42); the XmaI site is underlined), or gp64(154)-f (5'-GACTCCCGGGGCGCACACACGTGCAACAAATCG-3': (SEQ ID NO: 43); the XmaI site is underlined), or gp64(201)-f (5'-GACTCCCGGGGACACTGTGCTTCATCGAGACGGC-3': (SEQ ID NO: 44); the XmaI site is underlined), and GP64-r DralII (5'-GGGCACTTAGTGATATTGTCTATTACGGTTTCTAATC-3' (SEQ ID NO: 12); the DralII site is underlined). The obtained DNA fragments were cleaved with XmaI and DralII, and inserted into pCAP-HA1/Vietnam digested with XmaI and DralII. The constructed plasmids were designated pCAP-HA1/Vietnam/51, pCAP-HA1/Vietnam/101, pCAP-HA1/Vietnam/154, and pCAP-HA1/Vietnam/201.

**[0165]** The GenBank accession number of the amino acid sequence of the hemagglutinin of influenza virus A/H5N1/Vietnam/1203/2004 is AAW80717.

**(10) Construction of Transfer Vector Plasmids pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, pCAP-AH/520/467**

**[0166]** An artificial gene sequence (SEQ ID NO: 45) was prepared from the amino acid sequence of the HA region of the hemagglutinin of influenza virus A/H5N1/Anhui/1/05 by codon optimization using Gene Designer available from DNA2.0, Inc. Using this artificial sequence as a template, PCR was performed using AH17-F (5'-GACTCTGCAGGATCAGATCTGTATTGGGTACC-3' : (SEQ ID NO: 46); the PstI site is underlined), and AH345-R (5'-CGAT CCCGGGCTCTCTTCTCCTCCGCTCGC-3': (SEQ ID NO: 47); the XmaI site is underlined), or AH410-R (5'-CGAT CCCGGGCGGCCTCGAACTGGGTGTTTCATT-3': (SEQ ID NO: 48); the XmaI site is underlined), or AH473-R (5'-CGAT CCCGGGCGTCTCTGAGTTGAAGGCGCAC-3':(SEQ ID NO: 49); the XmaI site is underlined), or AH520-R (5'-CGAT CCCGGGCACCACTAATTCCTCTCGCTTC-3':(SEQ ID NO: 50); the XmaI site is underlined). The obtained DNA fragment was cleaved with PstI and XmaI, and inserted into pCAP-HA1/Anhui or pCAP-HA1/Anhui/467 digested with PstI and XmaI. The constructed plasmids were designated pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, and pCAP-AH/520/467.

**(11) Construction of Transfer Vector Plasmids pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, and pCAP-VN/520/467**

**[0167]** An artificial gene sequence (SEQ ID NO: 51) was prepared from the amino acid sequence of the HA region of the hemagglutinin of influenza virus A/H5N1/Vietnam/1203/2004 by codon optimization using Gene Designer available from DNA2.0, Inc. Using this artificial sequence as a template, PCR was performed using VN17-F (5'-GACTCTGCAGGATCAGATCTGTATCGGATATC-3': (SEQ ID NO: 52); the PstI site is underlined), and VN346-R (5'-

CGATCCCGGGCCTTTTTCTCCTCCGTTTCG-3': (SEQ ID NO: 53); the XmaI site is underlined), or VN410-R (5'-CGATCCCGGGCCTCAAACCTGCGTATTCATTTG-3': (SEQ ID NO: 54); the XmaI site is underlined), or VN473-R (5'-CGATCCCGGGCTCTAAGCTGGAGCCTGACTTTGTC-3': (SEQ ID NO: 55); the XmaI site is underlined), or VN520-R (5'-CGATCCCGGGCACTAATCTCCTCTCTTTAAGTC-3': (SEQ ID NO: 56); the XmaI site is underlined). The obtained DNA fragment was cleaved with PstI and XmaI, and inserted into pCAP-HA1/Anhui or pCAP-HA1/Anhui/467 digested with PstI and XmaI. The constructed plasmids were designated pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, and pCAP-VN/520/467.

**(12) Construction of Transfer Vector Plasmids pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, and pCAP-CO/205/467**

**[0168]** An artificial gene sequence (SEQ ID NO: 57) was prepared from the amino acid sequence of the CSP of *Plasmodium falciparum* 3D7 strain by codon optimization using Gene Designer available from DNA2.0, Inc. Using this artificial sequence as a template, PCR was performed using a pair of primers consisting of PfcSP\_opt-f (5'-GACTCTGCAGATGATGCGAAAATTGGCCATACTG-3': (SEQ ID NO: 58); the PstI site is underlined) and PfcSP\_opt-r (397) (5'-CGATCCCGGGCATTGAGGAACAGAAAGGAAAGAACCATG-3': (SEQ ID NO: 59); the XmaI site is underlined); PfcSP\_opt-f (19) (5'-GACTCTGCAGCTTTTTCAGGAATACCAGTGCTATGG-3': (SEQ ID NO: 60); (the PstI site is underlined) and PfcSP\_opt-r (373) (5'-CGATCCCGGGCCTTCTCCATCTTACAAATTTCTTTTCAATATCATTAGC-3': (SEQ ID NO: 61); the XmaI site is underlined); PfcSP\_opt-f (76) (5'-GACTCTGCAGGACGACGAAATAATGAGGACAACG-3' : (SEQ ID NO: 62); the PstI site is underlined) and PfcSP\_opt-r (373) (5'-CGATCCCGGGCCTTCTCCATCTTACAAATTTCTTTTCAATATCATTAGC-3': (SEQ ID NO: 61); the XmaI site is underlined); and PfcSP\_opt-f (205) (5'-GACTCTGCAGAATGCAAACCCAAATGCCAATCCAAACGC-3': (SEQ ID NO: 63); the PstI site is underlined) and PfcSP\_opt-r (373) (5'-CGATCCCGGGCCTTCTCCATCTTACAAATTTCTTTTCAATATCATTAGC-3': (SEQ ID NO: 61); the XmaI site is underlined). The obtained DNA fragments were cleaved with PstI and XmaI, and inserted into pCAP-HA1/Anhui or pCAP-HA1/Anhui/467 digested with PstI and XmaI. The constructed plasmids were designated pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, and pCAP-CO/205/467.

**(13) Construction of Transfer Vector Plasmids pCA64-HA1/Anhui and pCA64-PfCSP (A361E)**

**[0169]** Using the Triple Cut DNA of BacVector-2000 DNA (from Novagen), PCR was performed using gp64-p-f (5'-GACTCGGACCGGCCAGATAAAAATAATCTTATCAATTAAG-3': (SEQ ID NO: 64); the RsrII site is underlined) and gp64-p-r (5'-CGATACTAGTAGCACTGAGGCTTCTTATATACCCG-3': (SEQ ID NO: 65); the SpeI site is underlined). The obtained DNA fragment was cleaved with RsrII and SpeI, and inserted into pCAP-HA1/Anhui or pCAP-PfCSP(A361E) digested with RsrII and SpeI to construct transfer vector plasmids pCA64-HA1/Anhui and pCA64-PfCSP(A361E) enabling the expression of a fusion protein of HA1 antigen or PfCSP antigen and gp64 protein in mammalian and insect cells under the control of the desired dual promoter consisting of CAG promoter and gp64 promoter.

**(14) Construction of Transfer Vector Plasmids pCA39-HA1/Anhui and pCA39-PfCSP (A361E)**

**[0170]** Using the Triple Cut DNA of BacVector-2000 DNA (from Novagen), PCR was performed using vp39-p-f (5'-GACTCGGACCGCGTTCGTACAAATCGAAATATTGTTGTG-3': (SEQ ID NO: 66); the RsrII site is underlined) and vp39-p-r (5'-CGATACTAGTGTGATTGAGAAAGAAATCTCTTATTC-3': (SEQ ID NO: 67); the SpeI site is underlined). The obtained DNA fragment was cleaved with RsrII and SpeI, and inserted into pCAP-HA1/Anhui or pCAP-PfCSP(A361E) digested with RsrII and SpeI to construct transfer vector plasmids pCA39-HA1/Anhui and pCA39-PfCSP(A361E) enabling the expression of a fusion protein of HA1 antigen or PfCSP antigen and gp64 protein in mammalian and insect cells under the control of the desired dual promoter consisting of CAG promoter and vp39 promoter.

**(15) pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76/VSV, and pCAP-CO/205/VSV**

**[0171]** Using pVSV-G (from Clontech) as a template, PCR was performed using VSV-G-f (5'-GACTCCCCGGGCGTTTCCGAACATCCTCACATTCAAG - 3' (SEQ ID NO: 68); the XmaI site is underlined), VSV-G-r (5'-GACTCACTTAGTGCTTTCCAAGTCGGTTCATCTC-3': (SEQ ID NO: 69); the DraIII site is underlined). The obtained DNA

fragment was inserted into pCAP-CO/full, pCAP-CO/19, pCAP-CO/76, and pCAP-CO/205, each digested with XmaI and DraIII. The constructed plasmids were designated pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76/VSV, and pCAP-CO/205/VSV.

### Example 2: Recombinant Baculoviruses of the Present disclosure and Method for Production thereof

[0172]

- (1) Recombinant baculoviruses were produced using a kit for producing recombinant baculoviruses (BacVector-2000 Transfection Kit from Novagen) by co-transfecting BacVector-2000 DNA with each of the following transfer vectors constructed in Example 1: pCAP-PfCSP, pCAP-PfCSP/272, pCAP-PfCSP/467, pCAP-PfCSP(A361E), pCAP-PfCSP(A361E)/272, pCAP-PfCSP(A361E)/467, pCAP-PfCSP-76, pCAP-PfCSP-76/467, pCAP-PfCSP+209, pCAP-PfCSP+209/467, pCAP-PfCSP+76/209, pCAP-PfCSP+76/209/467, pCAP-HA1/Anhui, pCAP-HA1/Anhui/272, pCAP-HA1/Anhui/467, pCAP-HA1/Vietnam, pCAP-HA1/Vietnam/51, pCAP-HA1/Vietnam/101, pCAP-HA1/Vietnam/154, pCAP-HA1/Vietnam/201, pCAP-HA1/Vietnam/272, pCAP-HA1/Vietnam/467, pCAP-AH/345, pCAP-AH/345/467, pCAP-AH/410, pCAP-AH/410/467, pCAP-AH/473, pCAP-AH/473/467, pCAP-AH/520, pCAP-AH/520/467, pCAP-VN/346, pCAP-VN/346/467, pCAP-VN/410, pCAP-VN/410/467, pCAP-VN/473, pCAP-VN/473/467, pCAP-VN/520, pCAP-VN/520/467, pCAP-CO/full, pCAP-CO/full/467, pCAP-CO/19, pCAP-CO/19/467, pCAP-CO/76, pCAP-CO/76/467, pCAP-CO/205, pCAP-CO/205/467, pCA39-HA1/Anhui, pCA64-HA1/Anhui, pCA39-PfCSP(A361E), pCA64-PfCSP(A361E), pCAP-CO/full/VSV, pCAP-CO/19/VSV, pCAP-CO/76/VSV, pCAP-CO/205/VSV, pDual-Pfs25-PfCSP-gp64, pDual-PfMSP1-PfCSP-gp64 into Sf9 cells. The resulting recombinant baculoviruses were designated AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-PfCSP(A361E), AcNPV-CAP-PfCSP(A361E)/272, AcNPV-CAP-PfCSP(A361E)/467, AcNPV-CAP-PfCSP-76, AcNPV-CAP-PfCSP-76/467, AcNPV-CAP-PfCSP+209, AcNPV-CAP-PfCSP+209/467, AcNPV-CAP-PfCSP+76/209, AcNPV-CAP-PfCSP+76/209/467, AcNPV-CAP-HA1/Anhui, AcNPV-CAP-HA1/Anhui/272, AcNPV-CAP-HA1/Anhui/467, AcNPV-CAP-HA1/Vietnam, AcNPV-CAP-HA1/Vietnam/51, AcNPV-CAP-HA1/Vietnam/101, AcNPV-CAP-HA1/Vietnam/154, AcNPV-CAP-HA1/Vietnam/201, AcNPV-CAP-HA1/Vietnam/272, AcNPV-CAP-HA1/Vietnam/467, AcNPV-CAP-AH/345, AcNPV-CAP-AH/345/467, AcNPV-CAP-AH/410, AcNPV-CAP-AH/410/467, AcNPV-CAP-AH/473, AcNPV-CAP-AH/473/467, AcNPV-CAP-AH/520, AcNPV-CAP-AH/520/467, AcNPV-CAP-VN/346, AcNPV-CAP-VN/346/467, AcNPV-CAP-VN/410, AcNPV-CAP-VN/410/467, AcNPV-CAP-VN/473, AcNPV-CAP-VN/473/467, AcNPV-CAP-VN/520, AcNPV-CAP-VN/520/467, AcNPV-CAP-CO/full, AcNPV-CAP-CO/full/467, AcNPV-CAP-CO/19, AcNPV-CAP-CO/19/467, AcNPV-CAP-CO/76, AcNPV-CAP-CO/76/467, AcNPV-CAP-CO/205, AcNPV-CAP-CO/205/467, AcNPV-CA39-HA1/Anhui, AcNPV-CA64-HA1/Anhui, AcNPV-CA39-PfCSP(A361E), AcNPV-CA64-PfCSP(A361E), AcNPV-CAP-CO/full/VSV, AcNPV-CAP-CO/19/VSV, AcNPV-CAP-CO/76/VSV, AcNPV-CAP-CO/205/VSV, AcNPV-Dual-Pfs25-PfCSP-gp64, and AcNPV-Dual-PfMSP1-PfCSP-gp64.

### Example 3: Test of Expression of Vaccine Antigen from Recombinant Baculovirus of the Present disclosure in Insect Cells

[0173] Sf9 cells were cultured at a concentration of  $1 \times 10^5$  cells per well in a 48-well plate (from Corning), and infected with baculoviruses AcNPV-CAP-PfCSP, AcNPV-CAP-HA1/Anhui, and AcNPV-CAP-HA1/Vietnam obtained in Example 2, or a wild-type baculovirus AcNPV-WT as a control at an infection multiplicity of about 0.1. After 5 days, the culture supernatant was removed from each well, and then Sample Buffer Solution (+2ME, x2) (from Wako) was added in an amount of 0.05 mL per well to completely lyse the cells. The cell lysate was heated at 100°C for 5 minutes, and electrophoresed on 7.5% SDS-PAGE. After electrophoresis, the protein was transferred to a PVDF membrane (Immobilon-P from Millipore), and blocking was performed at 4°C overnight by immersing the membrane in 2.5% Skim Milk/SuperBlock (from Pierce). The membrane to which the protein of Sf9 cells infected with each baculovirus had been transferred was incubated with an anti-gp64 antibody (AcV5 from eBioScience) as the primary antibody, and then incubated with a HRP-labeled goat anti-mouse IgG (H+L) antibody (from BioRad) as the second antibody. Color was developed with an ECLplus Western Blotting Detection kit (from GE Healthcare) to detect the protein band. FIG. 1 shows the results.

[0174] FIG. 1 shows Western blotting analysis showing the expression of fusion antigens in insect cells from recombinant baculoviruses containing PfCSP gene of human malaria, HA1 gene of influenza virus H5N1/Anhui/1/05 strain, and HA1 gene of H5N1/Vietnam/1203/04 strain. In FIG. 1, Lane 1 shows the band of a wild-type baculovirus (AcNPV-WT); Lane 2 shows the band of a recombinant baculovirus (AcNPV-CAP-PfCSP) containing PfCSP gene and full-length gp64 gene inserted downstream of the

dual promoter used in the present invention Lane 3 shows the band of a recombinant baculovirus (AcNPV-CAP-HA1/Anhui) containing HA1 gene of influenza virus H5N1/Anhui/1/05 strain and full-length gp64 gene inserted downstream of the dual promoter used in the present invention; and Lane 4 shows the band of a recombinant baculovirus ((AcNPV-CAP-HA1/Vietnam) containing HA1 gene of influenza virus H5N1/Vietnam/1203/04 strain and full-length gp64 gene inserted downstream of the dual promoter used in the present invention.

**[0175]** As shown in Lanes 2, 3 and 4 of FIG. 1, a band corresponding to the expressed fusion product of an immunogenic foreign antigen gene and gp64 gene was observed in the recombinant baculoviruses having an antigen gene and gp64 gene fused and expressed downstream of the dual promoter used in the present invention.

**[0176]** The above results confirmed that when using the recombinant virus used in the present invention, an immunogenic foreign antigen gene and gp64 gene can be fused and expressed as an expressed fusion product in insect cells.

**Example 4: Test of Identification of Fusion Antigen in Vaccine Antigen Presented on Viral Particle (Virion) of Recombinant Baculovirus of the Present disclosure**

**[0177]** Sf9 cells were cultured to a concentration of  $1 \times 10^7$  cells per 150 mm cell culture plate (from Sumilon), and infected with each of the above-mentioned baculoviruses at an infection multiplicity of about 0.1. After 7 days, the medium was centrifuged at 3,000 xg at 4°C for 15 minutes twice, and the virus solution was layered over a 25% sucrose solution, and centrifuged using an ultracentrifuge at 25,000 rpm at 4°C for 90 minutes to yield viral particles. 0.05 mL of Sample Buffer Solution (+2ME, x2) (from Wako) was added to 0.05 mL each of the virus concentrates ( $1 \times 10^8$  PFU/mL) of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, AcNPV-CAP-PfCSP/467, AcNPV-CAP-HA1/Vietnam, and AcNPV-WT collected by ultracentrifugation. The resulting mixtures were heated at 100°C for 5 minutes, and electrophoresed on 7.5% SDS-PAGE. After the electrophoresis, the obtained proteins were transferred to PVDF membranes (Immobilon-P from Millipore), and immersed in 2.5% Skim Milk/SuperBlock (from Pierce) to perform blocking at 4°C overnight. The membranes to which the virus solutions of AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/272, and AcNPV-CAP-PfCSP/467 had been transferred were incubated with an anti-PfCSP antibody (2A10, MR-4) as the primary antibody, and then incubated with a HRP-labeled goat anti-mouse IgG (H+L) antibody (from BioRad) as the second antibody. The membrane to which the virus solution of AcNPV-CAP-HA1/Vietnam had been transferred was incubated with an anti-H5N1 antibody (IT-003-005 from Immune Technology) as the primary antibody, and then incubated with a HRP-labeled goat anti-rabbit IgG antibody (from GE Healthcare) as the second antibody. Color was developed with an ECLplus Western Blotting Detection kit (from GE Healthcare) to detect the bands of the proteins. FIG. 2 shows the results.

**[0178]** FIG. 2 (A) shows Western blotting analysis showing the expression of the CSP gene (PfCSP) of human malaria in viral particles of recombinant baculoviruses prepared using recombinant transfer vectors. Lane 1, the left side lane of FIG. 2 (A), shows the band of a recombinant baculovirus (AcNPV-CAP-PfCSP) containing PfCSP gene and full-length gp64 gene inserted downstream of the dual promoter used in the invention; Lane 2 shows the band of a recombinant baculovirus (AcNPV-CAP-PfCSP/272) containing PfCSP gene and partial-length gp64 gene (241 amino acid residues from the C terminus) inserted downstream of the dual promoter used in the invention; Lane 3 shows the band of a recombinant baculovirus (AcNPV-CAP-PfCSP/467) containing PfCSP gene and partial-length gp64 gene (46 amino acid residues from the C terminus) inserted downstream of the dual promoter used in the invention. The baculoviruses were electrophoresed, and the presence of an expressed fusion product of the PfCSP gene and gp64 gene was checked. A strong band, indicating the presence of a fusion antigen of PfCSP gene and gp64 gene in the recombinant viral particles, was detected in all the lanes of FIG. 2 (A).

**[0179]** FIG. 2 (B) shows Western blotting analysis showing the expression of H5N1/HA1 gene in viral particles of recombinant baculoviruses prepared using recombinant transfer vectors. Lane 1, left side lane of FIG. 2 (B), shows the results obtained using the infected AcNPV-WT cell lysate prepared in Example 3; Lane 2 shows the results obtained using the AcNPV-CAP-HA1/Anhui-infected cell lysate prepared in Example 3; Lane 3 shows the results obtained using the AcNPV-CAP-HA1/Vietnam-infected cell lysate prepared in Example 3; and Lane 4 shows the results of viral particles of a wild-type baculovirus (AcNPV-WT); Lane 5 shows the results of viral particles of a recombinant baculovirus (AcNPV-CAP-HA1/Vietnam) containing the HA1 gene of influenza virus H5N1/Vietnam/1203/04 strain and full-length gp64 gene inserted downstream of the dual promoter used in the present invention; and Lane 6 shows the results of purified HA antigen of H5N1 (IT-003-0053p from Immune Technology). The baculoviruses were electrophoresed, and the presence of an expressed fusion product of the PfCSP gene and gp64 gene was checked. A strong band, indicating the presence of a fusion antigen of HA1 gene of H5N1/Vietnam/1203/04 and gp64 gene in the recombinant viral particles, was detected in Lane 5 of FIG. 2(B).

**[0180]** The above results of Example 4 show that a foreign gene having the desired immunogenicity and gp64 gene can be

fused and expressed in recombinant viral particles of the recombinant baculovirus of the present disclosure produced by using the recombinant transfer vector of the present disclosure.

#### **Example 5: Test of Expression of Vaccine Antigen from Recombinant Baculovirus of the Present disclosure in Mammals**

[0181] HepG2 cells were infected with AcNPV-Dual-PfMSP1-PfCSP at an infection multiplicity of 1. After 48 hours, the culture supernatant was removed, and the plate was rinsed with PBS three times. An acetone/ethanol solution (a mixed ratio of 7:3) cooled to -20°C was added to immobilize the cells at -20°C for 5 minutes. A 5% normal goat serum (from Sigma) was added to perform blocking at room temperature for 1 hour. To detect the expression of PfCSP, an anti-PfCSP antibody (2A10, MR-4) labeled with Alexa Flour 594 was added; and to detect the expression of PfMSP-1<sub>19</sub>, an anti-PfMSP-1<sub>19</sub> antibody (5.2, MR-4) and then an anti-mouse antibody labeled with FITC were added. After incubation, the reacted cells were detected under a fluorescence microscope.

[0182] FIG. 3 shows the results.

[0183] FIG. 3 shows HepG2 cells stained with a fluorescence-labeled antibody, which indicates that an antigen has been expressed from a recombinant baculovirus containing a fusion gene of the PfMSP1 gene and PfCSP gene in the HepG2 cells. The results of FIG. 3 (A) confirmed that a PfCSP antigen has been expressed. The results of FIG. 3 (B) confirmed that a PfMSP-1<sub>19</sub> antigen has been expressed. It was thus confirmed that fusion antigens can be expressed in mammalian cells. The results of FIG. 3 (A) and (B) clearly show that the recombinant baculovirus produced by using the transfer vector containing the dual promoter of the present disclosure can express the desired antigen in mammalian cells.

[0184] This suggests that when the recombinant baculovirus produced using the recombinant transfer vector of the present disclosure is administered to humans and other mammals, the virus particles enter the mammalian cells, and a mammalian promoter operates to produce a fusion product of the desired foreign antigen gene and gp64 gene in the mammalian cells, thus inducing the acquired immunity.

#### **Example 6: Induction of Antibody by PfCSP Antigen Recombinant Virus and H5N1/HA1 Antigen Recombinant Virus**

##### **1. Inoculation of Virus Solution**

[0185] Virus solutions of AcNPV-WT, AcNPV-CAP-PfCSP, AcNPV-CAP-PfCSP/467, AcNPV-CAP-HA1/Anhui, and AcNPV-CAP-HA1/Vietnam concentrated by ultracentrifugation were inoculated into the thigh muscles of BALB/c female mice in an amount of  $1 \times 10^8$  PUF twice at two week-intervals.

##### **2. Measurement of Antibody Titers**

[0186] The mice were euthanized two weeks after the final immunization, and sera were collected from the mice and used for measuring antigen-specific antibody titers. Induction of PfCSP antigen-specific antibody titers by AcNPV-CAP-PfCSP and AcNPV-CAP-PfCSP/467 was measured by ELISA using a plate on which (NANP)<sub>4</sub>NVDPC peptide (from Sigma), i.e., B-cell epitope of PfCSP, had been immobilized. Induction of H5N1/HA antigen-specific antibody titers by AcNPV-CAP-HA1/Anhui and AcNPV-CAP-HA1/Vietnam was measured by ELISA using a plate on which purified HA antigen of H5N1 virus (IT-003-005p from Immune Technology) had been immobilized. The absorbance at OD450 nm was measured using MaxiSorp (from NUNC) as the ELISA plate, HRP-labeled goat anti-mouse IgG (H&L) antibody (from American Qualex) as the secondary antibody, and TMB (from Calbiochem) for the color reaction.

[0187] FIG. 4 shows the results.

[0188] FIG. 4 (A) is a graph plotting the average absorbance of each group at OD450 nm obtained when the mouse sera were subjected to two-fold serial dilution from 800-fold to 102,400-fold dilutions. In the groups inoculated with PBS and AcNPV-WT, whose sera contained no antibody to the antigen, absorbance of even the 800-fold dilutions was 0.1 or less, indicating low

reactivity. In contrast, in the groups inoculated with AcNPV-CAP-PfCSP and AcNPV-CAF-PfCSP/467, absorbance of the 800-fold dilutions was 1.138 and 1.878, respectively, indicating strong reactivity and clearly showing that antigen-specific antibodies were induced. FIG. 4 (B) is a graph plotting the average absorbance of each group at OD450 nm obtained when the mouse sera were subjected to two-fold serial dilution from 400-fold to 25,600-fold dilutions. In the groups inoculated with PBS and AcNPV-WT, whose sera contained no antibody to the antigen, absorbance of the 400-fold dilutions was 0.1 or less, indicating low reactivity. In contrast, in the group inoculated with AcNPV-CAP-HA1/Anhui and AcNPV-CAP-HA1/Vietnam, absorbance of the 3,200-fold dilution was 1.551 and 2.503, respectively, indicating strong reactivity and clearly showing that antigen-specific antibodies were induced. FIG. 4 clearly shows that the recombinant baculovirus produced using a transfer vector containing the dual promoter used in the present invention can induce an antibody to the desired antigen in mammals.

### 3. Measurement of Neutralization Value

**[0189]** A hemagglutination inhibition (HI) test was performed using a mouse serum inoculated with AcNPV-CAP-HA1/Anhui. More specifically, the test was performed according to the method described in the instructions packaged with an influenza HI reagent "Seiken" (from Denka Seiken Co., Ltd.), using purified HA antigen of H5N1 virus (IT-003-0053p from Immune Technology). Absorption of non-specific agglutinins was performed using erythrocyte, and removal of non-specific agglutination inhibitors was performed using RDE (II) of "Seiken" (from Denka Seiken Co., Ltd.). The HI test was performed in the following manner. 0.025 mL of 10-fold diluted antiserum in a 96-well plate was subjected to 2-fold serial dilution using a diluent. To each well of the 96-well plate containing the diluted antiserum, 0.025 mL of HA antigen of H5N1 virus diluted to obtain an HA titer of 4 per 0.025 mL thereof was added. The plate was allowed to stand at room temperature for 30 minutes. 0.05 mL of an erythrocyte suspension for the reaction was added and stirred well. The mixture was allowed to stand at room temperature for 60 minutes. The final dilution of the test sample at which hemagglutination was completely inhibited was defined as the HI antibody titer.

**[0190]** The results show that the sera inoculated with PBS and AcNPV-WT had HI antibody titers of 10 or less, whereas the serum inoculated with AcNPV-CAP-HA1/Anhui had an HI antibody titer of 40.

**[0191]** The results seem to indicate that when administered to humans and other mammals, the recombinant baculovirus produced from the recombinant transfer vector of the present disclosure can induce an antibody effective to the desired foreign antigen gene, thus providing vaccine effects.

### Sequence Listing Free Text

#### **[0192]**

SEQ ID NOS: 1 and 2 are the sequences of primers phsp65-F1 and phsp65-R1 for PCR of genomic DNA of *M. tuberculosis* H37Rv.

SEQ ID NOS: 3 and 4 are the sequences of primers phsp65-F2 and phsp65-R2 for PCR with pcDNA-hps65 as a template.

SEQ ID NOS: 5 and 6 are the sequences of primers pPolh-F2 and pgp64-R2 for PCR with pBACsurf-1 as a template to produce a gp64 gene DNA fragment.

SEQ ID NOS: 7 and 8 are the sequences of primers HA-f and HA-r for PCR to produce an influenza virus HA gene fragment.

SEQ ID NOS: 9 and 10 are the sequences of primers pHA-F1 and pHA-R1 for PCR with pCR-Blunt-HA as a template.

SEQ ID NOS: 11 and 12 are the sequences of primers Polh-f Rsrll and GP64-r Dralll for PCR with pBACsurf-HA1 as a template.

SEQ ID NOS: 13 and 14 are the sequences of primers NP-f EcoRI and NP-r Cfr9I for RT-PCR of genomic RNA of influenza virus PR/8/34 strain.

SEQ ID NOS: 15, 16, and 12 are the sequences of primers gp64(272)-f, gp64(467)-f, and GP64-r Dralll for PCR of pCAP-H1N1/HA1-gp64.

SEQ ID NOS: 17 and 18 are the sequences of primers PfCSP-f(19) and PfCSP-r(373) for PCR with *P. falciparum* genomic DNA as a template.

SEQ ID NOS: 19 and 20 are the sequences of primers pAMA-F1 and pAMA1-R1 for PCR with *P. berghei* genomic DNA as a

template.

SEQ ID NOS: 21 and 22 are the sequences of primers pPcSP-F1 and pPcSP-R1 for PCR with *P. falciparum* genomic DNA as a template.

SEQ ID NOS: 23 and 24 are the sequences of primers pPfs25-F1 and pPfs25-R2 for PCR with *P. falciparum* genomic DNA as a template.

SEQ ID NOS: 25 and 26 are the sequences of primers pPmSP119-F1 and pPmSP119-R2 for PCR with *P. falciparum* genomic DNA as a template.

SEQ ID NOS: 17 and 27 are the sequences of primers PfcSP-f(19) and PfcSP-r(373 A361E) for PCR with pCAP-PfcSP as a template.

SEQ ID NOS: 28 and 27 are the sequences of primers PfcSP-f(76) and PfcSP-r(373 A361E) for PCR with pCAP-PfcSP as a template.

SEQ ID NO: 29 is the sequence of an artificial gene (PfcSP+) produced from the amino acid sequence of the PfcSP of *P. falciparum* 3D7 strain (in which, however, the A at the 361-position was replaced by E) using codons frequently used in Sf9 and human cells.

SEQ ID NOS: 30 and 31 are the sequences of primers PfcSP-f(+209) and PfcSP-r(+A361E) for PCR with PfcSP+ as a template.

SEQ ID NOS: 32, 33, 34 and 31 are the sequences of primers PfcSP-f(+76), PfcSP-r(+128), PfcSP-f(+209) BamHI, and PfcSP-r(+A361E) for PCR with PfcSP+ as a template.

SEQ ID NO: 35 is the sequence of an artificial gene produced from the amino acid sequence of the HA1 region of the hemagglutinin of influenza virus H5N1/Anhui/1/05 using codons frequently used in Sf9 and human cells.

SEQ ID NOS: 36 and 37 are the sequences of primers AH-F1 (5'-CAGTCTGCAGGACCAGATTTGCATC-3': (SEQ ID NO: 36); the PstI site is underlined) and AH-R4 (5'-CAGTCCCGGGCTCTCTTGCCTGC-3': (SEQ ID NO: 37); the XmaI site is underlined) for PCR with the artificial gene sequence of SEQ ID NO: 35 as a template.

SEQ ID NO: 38 is the sequence of an artificial gene produced from the amino acid sequence of the HA1 region of the hemagglutinin of influenza virus H5N1/Vietnam/1203/04 using codons frequently used in Sf9 and human cells.

SEQ ID NOS: 39 and 40 are the sequences of primers VN-F1 (5'-CAGTCTGCAGGACCAGATCTGTATC-3': (SEQ ID NO: 39); the PstI site is underlined), and VN-R4 (5'-CAGTCCCGGGCTCTCTTCTCCTGC-3': (SEQ ID NO: 40); the XmaI site is underlined) for PCR with the artificial gene sequence of SEQ ID NO: 38 as a template.

SEQ ID NOS: 41, 42, 43, 44, and 12 are the sequences of primers gp64(51)-f (5'-GACTCCCGGGGTTGAAATCACCATCGTGGAGACG-3': (SEQ ID NO: 41); the XmaI site is underlined), gp64(101)-f (5'-GACTCCCGGGGATTTGCTTATGTGGAGCATCAGG-3': (SEQ ID NO: 42); the XmaI site is underlined), gp64(154)-f (5'-GACTCCCGGGGCGCACCACACGTGCAACAAATCG-3': (SEQ ID NO: 43); the XmaI site is underlined), gp64(201)-f (5'-GACTCCCGGGGACACTGTGCTTCATCGAGACGGC-3': (SEQ ID NO: 44); the XmaI site is underlined), and GP64-r DralI (5'-GGGCACTTAGTGATATTGTCTATTACGGTTTCTAATC-3' (SEQ ID NO: 12); the DralI site is underlined).

SEQ ID NO: 45 is the sequence of an artificial gene produced from the amino acid sequence of the HA1 region of the hemagglutinin of influenza virus H5N1/Anhui/1/05 by codon optimization using Gene Designer available from DNA2.0, Inc.

SEQ ID NOS: 46, 47, 48, 49, and 50 are the sequences of AH17-F (5'-GACTCTGCAGGATCAGATCTGTATTGGGTACC-3': (SEQ ID NO: 46); the PstI site is underlined), and AH345-R (5'-CGAT CCCGGGCTCTCTTTCTCCTCCGCTCGC-3': (SEQ ID NO: 47); the XmaI site is underlined), AH410-R (5'-CGAT CCCGGGGCGCCTCGAACTGGGTGTTTCATT-3': (SEQ ID NO: 48); the XmaI site is underlined), AH473-R (5'-CGATCCCGGGCGTCTCTGAGTTGAAGGCGCAC-3': (SEQ ID NO: 49); the XmaI site is underlined), and AH520-R (5'-CGATCCCGGGCACCACTAATTTCTCCTCCTC-3': (SEQ ID NO: 50); the XmaI site is underlined) for PCR with the artificial gene sequence of SEQ ID NO: 45 as a template.

SEQ ID NO: 51 is the sequence of an artificial gene produced from the amino acid sequence of the HA1 region of the hemagglutinin of influenza virus H5N1/Vietnam/1203/04 by codon optimization using Gene Designer available from DNA2.0, Inc.

SEQ ID NOS: 52, 53, 54, 55, and 56 are the sequences of primers VN17-F (5'-GACTCTGCAGGATCAGATCTGTATCGGATATC-3': (SEQ ID NO: 52); the PstI site is underlined), and VN346-R (5'-CGATCCCGGGCCCGCTTTTCTCCTCCTCCGTTTCG-3' : (SEQ ID NO: 53); the XmaI site is underlined), VN410-R (5'-CGATCCCGGGCCTCAAACCTGCGTATTCATTTG-3' : (SEQ ID NO: 54); the

XmaI site is underlined), VN473-R (5'-CGATCCCGGGCTCTAAGCTGGAGCCTGACTTTGTC-3': (SEQ ID NO: 55); the XmaI site is underlined), and VN520-R (5'-CGATCCCGGGCACTAATCTCCTCTCTTTAAGTC-3': (SEQ ID NO: 56); the XmaI site is underlined) for PCR with the artificial gene sequence of SEQ ID NO: 51 as a template.

SEQ ID NO: 57 is the sequence of an artificial gene produced from the amino acid sequence of the CSP of *Plasmodium falciparum* 3D7 strain by codon optimization using Gene Designer available from DNA2.0, Inc.

SEQ ID NOS: 58, 59, 60, 61, and 62 are the sequences of primers PfcSP\_opt-f (5'-GACTCTGCAGATGATGCGAAAATTGGCCATACTG-3': (SEQ ID NO: 58); the PstI site is underlined), PfcSP\_opt-r (397) (5'-CGATCCCGGGCATTGAGGAACAGAAAGGAAAGAACCATG-3': (SEQ ID NO: 59); the XmaI site is underlined), PfcSP\_opt-f (19) (5'-GACTCTGCAGCTGTTTCAGGAATACCAGTGCTATGG-3': (SEQ ID NO: 60); (the PstI site is underlined), PfcSP\_opt-r(373) (5'-CGATCCCGGGCCTTCTCCATCTTACAAATTTCTTTTCAATATCATTAGC -3': (SEQ ID NO: 61); (the XmaI site is underlined), PfcSP\_opt-f (76) (5'-GACTCTGCAGGACGACGGAAATAATGAGGACAACG-3': (SEQ ID NO: 62); the PstI site is underlined), and PfcSP\_opt-f (205) (5'-GACTCTGCAGAAATGCAAACCCAAATGCCAATCCAACGC-3': (SEQ ID NO: 63) ; the PstI site is underlined) for PCR with the artificial gene sequence of SEQ ID NO: 57 as a template.

SEQ ID NOS: 64, 65, 66, and 67 are the sequences of primers gp64-p-f (5'-GACTCGGACCGGCCAGATAAAAATAATCTTATCAATTAAG-3': (SEQ ID NO: 64); the RsrII site is underlined), gp64-p-r (5'-CGATACTAGTAGCACTGAGGCTTCTTATATACCCG-3': (SEQ ID NO: 65); the SpeI site is underlined), and vp39-p-f (5'-GACTCGGACCGCGTCGTACAAATCGAAATATTGTTGTG-3': (SEQ ID NO: 66) ; the RsrII site is underlined), and vp39-p-r (5'-CGATACTAGTGTGATTGAGAAAGAAATCTTATTC-3': (SEQ ID NO: 67); the SpeI site is underlined) for PCR with Baculovirus genomic DNA as a template.

SEQ ID NOS: 68 and 69 are the sequences of primers VSV-G-f (5'-GACTCCCCGGGCGTTTGAACATCCTCACATTCAAG -3' (SEQ ID NO: 68); the XmaI site is underlined), and VSV-G-r (5'-GACTCACCTAGTGCTTTCCAAGTCGGTTTCATCTC-3': (SEQ ID NO: 69); the DraIII site is underlined) for PCR with pVSV-G as a template.

## SEQUENCE LISTING

### [0193]

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OTSUKA PHARMACEUTICAL CO., LTD.

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<223> primer PFCSP-r(373 A361E)

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<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PFCSP-f(76)

<400> 28

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<210> 29

<211> 1194

<212> DNA

<213> Plasmodium falciparum

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<223> primer PfcSP-r(+ A361 E)

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<223> primer PfcSP-r(+128)

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<210> 34

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<210> 35  
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 ctctogagac tggacggcgt gaaacccctc atcctgagag attgttctgt ggccggatgg 180  
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<400> 36  
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<210> 38  
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<212> DNA

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<400> 38

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<223> primer gp64(101)-f

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<223> primer gp64(154)-f

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<210> 44

<211> 34

<212> DNA

<213> Artificial Sequence

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<223> primer gp64(201)-f

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<211> 1701

<212> DNA

<213> influenza virus

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<211> 33

<212> DNA

<213> Artificial Sequence

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<223> primer AH410-R

<400> 48

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<210> 49

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer AH473-R

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<210> 50

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> primer AH520-R

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<211> 1704

<212> DNA

<213> influenza virus

<400> 51

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<223> primer VN17-F

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<212> DNA

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<223> primer VN410-R

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<210> 57

<211> 1191

<212> DNA

<213> Plasmodium falciparum

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 aatgcaaatc ccaatgcaaa tccaatgcg aacccgaatg ctaaccctaa tgcaaatcct 780  
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 caggacata atatgcotaa tgaccctaac aggaacgttg atgagaacgc gaatgcgaac 900  
 totgtgtaa agaacaacaa caatgaagag ccoctcgata aacatattaa ggagtatctg 960  
 aataagatcc agaactcctt gtctaccgaa tggccccct gttctgtgac gtgtggtaac 1020  
 ggaatccagg taaggatcaa acccggcagt gccacaacgc caaaggacga gctcgattac 1080  
 gctaatgata ttgaaaagaa aatttgaag atggagaagt gcagctcgtt attcaatgtg 1140  
 gtaacacgct caattggcct catcatggtt ctttcttctt tgttctctaa t 1191

<210> 58

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PFCSP\_opt-f

<400> 58

gactctgcag atgatgcgaa aattggccat actg 34

<210> 59

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PFCSP\_opt-r(397)

<400> 59

cgatcccggg cattgaggaa cagaaaggaa agaaccatg 39

<210> 60

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PFCSP\_opt-f(19)

<400> 60

gactctgcag ctgtttcagg aataccagtg ctatgg 36

<210> 61

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PfcSP\_opt-r(373)

<400> 61  
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 <213> Artificial Sequence

<220>

<223> primer PfcSP\_opt-f(76)

<400> 62  
 gactctgag gacgacgaa ataatgagga caacg 35

<210> 63  
 <211> 39  
 <212> DNA  
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<220>

<223> primer PfcSP\_opt-f(205)

<400> 63  
 gactctgag aatgcaaacc caaatgcaa tccaaacgc 39

<210> 64  
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 <213> Artificial Sequence

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<223> primer gp64-p-f

<400> 64  
 gactcggacc ggccagataa aaataatctt atcaattaag 40

<210> 65  
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 <212> DNA  
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<223> primer gp64-p-r

<400> 65  
 cgatactagt agcactgagg ctcttatat acccg 35

<210> 66  
 <211> 38  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> primer vp39-p-f

<400> 66  
 gactcggacc gcgtcgtaca aatcgaata ttgtgtg 38

<210> 67

<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer gp64-p-r

<400> 67  
cgatactagt gtgattgaga aagaaatctc ttattc        36

<210> 68  
<211> 35  
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<213> Artificial Sequence

<220>  
<223> primer VSV-G-f

<400> 68  
gactccccgg gcggtcgaac atcctcacat tcaag        35

<210> 69  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer VSV-G-r

<400> 69  
gactcactta gtgcttcca agtcggtca tctc        34

<210> 70  
<211> 397  
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<213> PfCSP protein XP\_001351122

<400> 70

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

Glu Ala Leu Phe Gln Glu Tyr Gln Cys Tyr Gly Ser Ser Ser Asn Thr  
20 25 30

Arg Val Leu Asn Glu Leu Asn Tyr Asp Asn Ala Gly Thr Asn Leu Tyr  
35 40 45

Asn Glu Leu Glu Met Asn Tyr Tyr Gly Lys Gln Glu Asn Trp Tyr Ser  
50 55 60

Leu Lys Lys Asn Ser Arg Ser Leu Gly Glu Asn Asp Asp Gly Asn Asn  
65 70 75 80

Glu Asp Asn Glu Lys Leu Arg Lys Pro Lys His Lys Lys Leu Lys Gln  
85 90 95

Pro Ala Asp Gly Asn Pro Asp Pro Asn Ala Asn Pro Asn Val Asp Pro  
100 105 110

Asn Ala Asn Pro Asn Val Asp Pro Asn Ala Asn Pro Asn Val Asp Pro  
115 120 125

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro  
130 135 140

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro  
145 150 155 160

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro

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

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Met Glu Lys Ile Val Leu Leu Leu Ala Ile Val Ser Leu Val Lys Ser  
 1 5 10 15

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

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile  
 35 40 45

Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys  
 50 55 60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn  
 65 70 75 80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val  
 85 90 95

Glu Lys Ala Asn Pro Ala Asn Asp Leu Cys Tyr Pro Gly Asn Phe Asn  
 100 105 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu  
 115 120 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asp His Glu Ala Ser  
 130 135 140

Ser Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Thr Pro Ser Phe Phe  
 145 150 155 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Asn Thr Tyr Pro Thr Ile  
 165 170 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Ile Leu Trp  
 180 185 190

Gly Ile His His Ser Asn Asp Ala Ala Glu Gln Thr Lys Leu Tyr Gln  
 195 200 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg  
 210 215 220

Leu Val Pro Lys Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly  
 225 230 235 240

Arg Met Asp Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn  
 245 250 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile  
 260 265 270

Val Lys Lys Gly Asp Ser Ala Ile Val Lys Ser Glu Val Glu Tyr Gly  
 275 280 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Ile Gly Ala Ile Asn Ser Ser  
 290 295 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys  
 305 310 315 320

Tyr Val Lys Ser Asn Lys Leu Val Leu Ala Thr Gly Leu Arg Asn Ser  
 325 330 335

Pro Leu Arg Glu Arg Arg Arg Lys Arg Gly Leu Phe Gly Ala Ile Ala  
 340 345 350

Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly  
 355 360 365

Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu  
 370 375 380

Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile  
 385 390 395 400

Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn  
 405 410 415

Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly  
 420 425 430

Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu  
 435 440 445

Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr  
 450 455 460

Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn  
 465 470 475 480

Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser  
 485 490 495

Val Arg Asn Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg  
 500 505 510

Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Thr  
 515 520 525

Tyr Gln Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu  
 530 535 540

Ala Ile Met Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser  
 545 550 555 560

Leu Gln Cys Arg Ile Cys Ile  
 565

<210> 72

<211> 568

<212> PRT

<213> HA/A/Vietnam/1203/2004 protein AAW80717

<400> 72

Met Glu Lys Ile Val Leu Leu Phe Ala Ile Val Ser Leu Val Lys Ser  
 1 5 10 15

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

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile  
 35 40 45

Leu Glu Lys Lys His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys  
 50 55 60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn  
 65 70 75 80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val  
 85 90 95

Glu Lys Ala Asn Pro Val Asn Asp Leu Cys Tyr Pro Gly Asp Phe Asn  
 100 105 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu  
 115 120 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Ser His Glu Ala Ser  
 130 135 140

Leu Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Lys Ser Ser Phe Phe  
 145 150 155 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Thr Tyr Pro Thr Ile  
 165 170 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp  
 180 185 190

Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Lys Leu Tyr Gln  
 195 200 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg  
 210 215 220

Leu Val Pro Arg Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly  
 225 230 235 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn  
 245 250 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile  
 260 265 270

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly  
 275 280 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser  
 290 295 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys  
 305 310 315 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser  
 325 330 335

Pro Gln Arg Glu Arg Arg Lys Lys Arg Gly Leu Phe Gly Ala Ile  
 340 345 350

Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr  
 355 360 365

Gly Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys  
 370 375 380

Glu Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser  
 385 390 395 400

Ile Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe  
 405 410 415

Asn Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp  
 420 425 430

Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met  
 435 440 445

Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu  
 450 455 460

Tyr Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly  
 465 470 475 480

Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu  
 485 490 495

Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala  
 500 505 510

Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly  
 515 520 525

Ile Tyr Gln Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala  
 530 535 540

Leu Ala Ile Met Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly  
 545 550 555 560

Ser Leu Gln Cys Arg Ile Cys Ile  
 565

<210> 73  
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 <212> PRT  
 <213> PfMSP1 protein XP\_001352170

<400> 73  
 Met Lys Ile Ile Phe Phe Leu Cys Ser Phe Leu Phe Phe Ile Ile Asn  
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Thr Gln Cys Val Thr His Glu Ser Tyr Gln Glu Leu Val Lys Lys Leu  
 20 25 30

Glu Ala Leu Glu Asp Ala Val Leu Thr Gly Tyr Ser Leu Phe Gln Lys  
 35 40 45

Glu Lys Met Val Leu Asn Glu Glu Ile Thr Thr Lys Gly Ala Ser  
 50 55 60

Ala Gln Ser Gly Ala Ser Ala Gln Ser Gly Ala Ser Ala Gln Ser Gly  
 65 70 75 80

Ala Ser Ala Gln Ser Gly Ala Ser Ala Gln Ser Gly Ala Ser Ala Gln  
 85 90 95

Ser Gly Thr Ser Gly Pro Ser Gly Pro Ser Gly Thr Ser Pro Ser Ser  
 100 105 110

Arg Ser Asn Thr Leu Pro Arg Ser Asn Thr Ser Ser Gly Ala Ser Pro  
 115 120 125

Pro Ala Asp Ala Ser Asp Ser Asp Ala Lys Ser Tyr Ala Asp Leu Lys  
 130 135 140

His Arg Val Arg Asn Tyr Leu Phe Thr Ile Lys Glu Leu Lys Tyr Pro  
 145 150 155 160

Glu Leu Phe Asp Leu Thr Asn His Met Leu Thr Leu Cys Asp Asn Ile  
 165 170 175

His Gly Phe Lys Tyr Leu Ile Asp Gly Tyr Glu Glu Ile Asn Glu Leu  
 180 185 190

Leu Tyr Lys Leu Asn Phe Tyr Phe Asp Leu Leu Arg Ala Lys Leu Asn  
 195 200 205

Asp Val Cys Ala Asn Asp Tyr Cys Gln Ile Pro Phe Asn Leu Lys Ile  
 210 215 220

Arg Ala Asn Glu Leu Asp Val Leu Lys Lys Leu Val Phe Gly Tyr Arg  
 225 230 235 240

Lys Pro Leu Asp Asn Ile Lys Asp Asn Val Gly Lys Met Glu Asp Tyr  
 245 250 255

Ile Lys Lys Asn Lys Thr Thr Ile Ala Asn Ile Asn Glu Leu Ile Glu  
 260 265 270

Gly Ser Lys Lys Thr Ile Asp Gln Asn Lys Asn Ala Asp Asn Glu Glu  
 275 280 285

Gly Lys Lys Lys Leu Tyr Gln Ala Gln Tyr Asp Leu Ser Ile Tyr Asn

290            295            300  
 Lys Gln Leu Glu Glu Ala His Asn Leu Ile Ser Val Leu Glu Lys Arg  
 305            310            315            320  
 Ile Asp Thr Leu Lys Lys Asn Glu Asn Ile Lys Lys Leu Leu Asp Lys  
                  325            330            335  
 Ile Asn Glu Ile Lys Asn Pro Pro Pro Ala Asn Ser Gly Asn Thr Pro  
                  340            345            350  
 Asn Thr Leu Leu Asp Lys Asn Lys Lys Ile Glu Glu His Glu Glu Lys  
                  355            360            365  
 Ile Lys Glu Ile Ala Lys Thr Ile Lys Phe Asn Ile Asp Ser Leu Phe  
                  370            375            380  
 Thr Asp Pro Leu Glu Leu Glu Tyr Tyr Leu Arg Glu Lys Asn Lys Lys  
 385            390            395            400  
 Val Asp Val Thr Pro Lys Ser Gln Asp Pro Thr Lys Ser Val Gln Ile  
                  405            410            415  
 Pro Lys Val Pro Tyr Pro Asn Gly Ile Val Tyr Pro Leu Pro Leu Thr  
                  420            425            430  
 Asp Ile His Asn Ser Leu Ala Ala Asp Asn Asp Lys Asn Ser Tyr Gly  
                  435            440            445  
 Asp Leu Met Asn Pro His Thr Lys Glu Lys Ile Asn Glu Lys Ile Ile  
                  450            455            460  
 Thr Asp Asn Lys Glu Arg Lys Ile Phe Ile Asn Asn Ile Lys Lys Lys  
 465            470            475            480  
 Ile Asp Leu Glu Glu Lys Asn Ile Asn His Thr Lys Glu Gln Asn Lys  
                  485            490            495  
 Lys Leu Leu Glu Asp Tyr Glu Lys Ser Lys Lys Asp Tyr Glu Glu Leu  
                  500            505            510  
 Leu Glu Lys Phe Tyr Glu Met Lys Phe Asn Asn Asn Phe Asn Lys Asp  
                  515            520            525  
 Val Val Asp Lys Ile Phe Ser Ala Arg Tyr Thr Tyr Asn Val Glu Lys  
                  530            535            540

Gln Arg Tyr Asn Asn Lys Phe Ser Ser Ser Asn Asn Ser Val Tyr Asn  
 545 550 555 560

Val Gln Lys Leu Lys Lys Ala Leu Ser Tyr Leu Glu Asp Tyr Ser Leu  
 565 570 575

Arg Lys Gly Ile Ser Glu Lys Asp Phe Asn His Tyr Tyr Thr Leu Lys  
 580 585 590

Thr Gly Leu Glu Ala Asp Ile Lys Lys Leu Thr Glu Glu Ile Lys Ser  
 595 600 605

Ser Glu Asn Lys Ile Leu Glu Lys Asn Phe Lys Gly Leu Thr His Ser  
 610 615 620

Ala Asn Gly Ser Leu Glu Val Ser Asp Ile Val Lys Leu Gln Val Gln  
 625 630 635 640

Lys Val Leu Leu Ile Lys Lys Ile Glu Asp Leu Arg Lys Ile Glu Leu  
 645 650 655

Phe Leu Lys Asn Ala Gln Leu Lys Asp Ser Ile His Val Pro Asn Ile  
 660 665 670

Tyr Lys Pro Gln Asn Lys Pro Glu Pro Tyr Tyr Leu Ile Val Leu Lys  
 675 680 685

Lys Glu Val Asp Lys Leu Lys Glu Phe Ile Pro Lys Val Lys Asp Met  
 690 695 700

Leu Lys Lys Glu Gln Ala Val Leu Ser Ser Ile Thr Gln Pro Leu Val  
 705 710 715 720

Ala Ala Ser Glu Thr Thr Glu Asp Gly Gly His Ser Thr His Thr Leu  
 725 730 735

Ser Gln Ser Gly Glu Thr Glu Val Thr Glu Glu Thr Glu Glu Thr Glu  
 740 745 750

Glu Thr Val Gly His Thr Thr Thr Val Thr Ile Thr Leu Pro Pro Thr  
 755 760 765

Gln Pro Ser Pro Pro Lys Glu Val Lys Val Val Glu Asn Ser Ile Glu  
 770 775 780

His Lys Ser Asn Asp Asn Ser Gln Ala Leu Thr Lys Thr Val Tyr Leu  
 785 790 795 800

Lys Lys Leu Asp Glu Phe Leu Thr Lys Ser Tyr Ile Cys His Lys Tyr  
 805 810 815

Ile Leu Val Ser Asn Ser Ser Met Asp Gln Lys Leu Leu Glu Val Tyr  
 820 825 830

Asn Leu Thr Pro Glu Glu Glu Asn Glu Leu Lys Ser Cys Asp Pro Leu  
 835 840 845

Asp Leu Leu Phe Asn Ile Gln Asn Asn Ile Pro Ala Met Tyr Ser Leu  
 850 855 860

Tyr Asp Ser Met Asn Asn Asp Leu Gln His Leu Phe Phe Glu Leu Tyr  
 865 870 875 880

Gln Lys Glu Met Ile Tyr Tyr Leu His Lys Leu Lys Glu Glu Asn His  
 885 890 895

Ile Lys Lys Leu Leu Glu Glu Gln Lys Gln Ile Thr Gly Thr Ser Ser  
 900 905 910

Thr Ser Ser Pro Gly Asn Thr Thr Val Asn Thr Ala Gln Ser Ala Thr  
 915 920 925

His Ser Asn Ser Gln Asn Gln Gln Ser Asn Ala Ser Ser Thr Asn Thr  
 930 935 940

Gln Asn Gly Val Ala Val Ser Ser Gly Pro Ala Val Val Glu Glu Ser  
 945 950 955 960

His Asp Pro Leu Thr Val Leu Ser Ile Ser Asn Asp Leu Lys Gly Ile  
 965 970 975

Val Ser Leu Leu Asn Leu Gly Asn Lys Thr Lys Val Pro Asn Pro Leu  
 980 985 990

Thr Ile Ser Thr Thr Glu Met Glu Lys Phe Tyr Glu Asn Ile Leu Lys  
 995 1000 1005

Asn Asn Asp Thr Tyr Phe Asn Asp Asp Ile Lys Gln Phe Val Lys  
 1010 1015 1020

Ser Asn Ser Lys Val Ile Thr Gly Leu Thr Glu Thr Gln Lys Asn  
 1025 1030 1035

Ala Leu Asn Asp Glu Ile Lys Lys Leu Lys Asp Thr Leu Gln Leu  
 1040 1045 1050

Ser Phe Asp Leu Tyr Asn Lys Tyr Lys Leu Lys Leu Asp Arg Leu  
1055 1060 1065

Phe Asn Lys Lys Lys Glu Leu Gly Gln Asp Lys Met Gln Ile Lys  
1070 1075 1080

Lys Leu Thr Leu Leu Lys Glu Gln Leu Glu Ser Lys Leu Asn Ser  
1085 1090 1095

Leu Asn Asn Pro His Asn Val Leu Gln Asn Phe Ser Val Phe Phe  
1100 1105 1110

Asn Lys Lys Lys Glu Ala Glu Ile Ala Glu Thr Glu Asn Thr Leu  
1115 1120 1125

Glu Asn Thr Lys Ile Leu Leu Lys His Tyr Lys Gly Leu Val Lys  
1130 1135 1140

Tyr Tyr Asn Gly Glu Ser Ser Pro Leu Lys Thr Leu Ser Glu Val  
1145 1150 1155

Ser Ile Gln Thr Glu Asp Asn Tyr Ala Asn Leu Glu Lys Phe Arg  
1160 1165 1170

Val Leu Ser Lys Ile Asp Gly Lys Leu Asn Asp Asn Leu His Leu  
1175 1180 1185

Gly Lys Lys Lys Leu Ser Phe Leu Ser Ser Gly Leu His His Leu  
1190 1195 1200

Ile Thr Glu Leu Lys Glu Val Ile Lys Asn Lys Asn Tyr Thr Gly  
1205 1210 1215

Asn Ser Pro Ser Glu Asn Asn Lys Lys Val Asn Glu Ala Leu Lys  
1220 1225 1230

Ser Tyr Glu Asn Phe Leu Pro Glu Ala Lys Val Thr Thr Val Val  
1235 1240 1245

Thr Pro Pro Gln Pro Asp Val Thr Pro Ser Pro Leu Ser Val Arg  
1250 1255 1260

Val Ser Gly Ser Ser Gly Ser Thr Lys Glu Glu Thr Gln Ile Pro  
1265 1270 1275

Thr Ser Gly Ser Leu Leu Thr Glu Leu Gln Gln Val Val Gln Leu

|   |      |      |
|---|------|------|
| 1280  | 1285 | 1290 |
| Gln Asn Tyr Asp Glu Glu Asp Asp Ser Leu Val Val Leu Pro Ile |      |      |
| 1295  | 1300 | 1305 |
| Phe Gly Glu Ser Glu Asp Asn Asp Glu Tyr Leu Asp Gln Val Val |      |      |
| 1310  | 1315 | 1320 |
| Thr Gly Glu Ala Ile Ser Val Thr Met Asp Asn Ile Leu Ser Gly |      |      |
| 1325  | 1330 | 1335 |
| Phe Glu Asn Glu Tyr Asp Val Ile Tyr Leu Lys Pro Leu Ala Gly |      |      |
| 1340  | 1345 | 1350 |
| Val Tyr Arg Ser Leu Lys Lys Gln Ile Glu Lys Asn Ile Phe Thr |      |      |
| 1355  | 1360 | 1365 |
| Phe Asn Leu Asn Leu Asn Asp Ile Leu Asn Ser Arg Leu Lys Lys |      |      |
| 1370  | 1375 | 1380 |
| Arg Lys Tyr Phe Leu Asp Val Leu Glu Ser Asp Leu Met Gln Phe |      |      |
| 1385  | 1390 | 1395 |
| Lys His Ile Ser Ser Asn Glu Tyr Ile Ile Glu Asp Ser Phe Lys |      |      |
| 1400  | 1405 | 1410 |
| Leu Leu Asn Ser Glu Gln Lys Asn Thr Leu Leu Lys Ser Tyr Lys |      |      |
| 1415  | 1420 | 1425 |
| Tyr Ile Lys Glu Ser Val Glu Asn Asp Ile Lys Phe Ala Gln Glu |      |      |
| 1430  | 1435 | 1440 |
| Gly Ile Ser Tyr Tyr Glu Lys Val Leu Ala Lys Tyr Lys Asp Asp |      |      |
| 1445  | 1450 | 1455 |
| Leu Glu Ser Ile Lys Lys Val Ile Lys Glu Glu Lys Glu Lys Phe |      |      |
| 1460  | 1465 | 1470 |
| Pro Ser Ser Pro Pro Thr Thr Pro Pro Ser Pro Ala Lys Thr Asp |      |      |
| 1475  | 1480 | 1485 |
| Glu Gln Lys Lys Glu Ser Lys Phe Leu Pro Phe Leu Thr Asn Ile |      |      |
| 1490  | 1495 | 1500 |
| Glu Thr Leu Tyr Asn Asn Leu Val Asn Lys Ile Asp Asp Tyr Leu |      |      |
| 1505  | 1510 | 1515 |

Ile Asn Leu Lys Ala Lys Ile Asn Asp Cys Asn Val Glu Lys Asp  
 1520 1525 1530

Glu Ala His Val Lys Ile Thr Lys Leu Ser Asp Leu Lys Ala Ile  
 1535 1540 1545

Asp Asp Lys Ile Asp Leu Phe Lys Asn Pro Tyr Asp Phe Glu Ala  
 1550 1555 1560

Ile Lys Lys Leu Ile Asn Asp Asp Thr Lys Lys Asp Met Leu Gly  
 1565 1570 1575

Lys Leu Leu Ser Thr Gly Leu Val Gln Asn Phe Pro Asn Thr Ile  
 1580 1585 1590

Ile Ser Lys Leu Ile Glu Gly Lys Phe Gln Asp Met Leu Asn Ile  
 1595 1600 1605

Ser Gln His Gln Cys Val Lys Lys Gln Cys Pro Glu Asn Ser Gly  
 1610 1615 1620

Cys Phe Arg His Leu Asp Glu Arg Glu Glu Cys Lys Cys Leu Leu  
 1625 1630 1635

Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val Glu Asn Pro Asn Pro  
 1640 1645 1650

Thr Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala Asp Ala Thr Cys  
 1655 1660 1665

Thr Glu Glu Asp Ser Gly Ser Ser Arg Lys Lys Ile Thr Cys Glu  
 1670 1675 1680

Cys Thr Lys Pro Asp Ser Tyr Pro Leu Phe Asp Gly Ile Phe Cys  
 1685 1690 1695

Ser Ser Ser Asn Phe Leu Gly Ile Ser Phe Leu Leu Ile Leu Met  
 1700 1705 1710

Leu Ile Leu Tyr Ser Phe Ile  
 1715 1720

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<211> 217

<212> PRT

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## REFERENCES CITED IN THE DESCRIPTION

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### Patent documents cited in the description

- [JP3366328B \[0011\]](#)
- [WO98011243A \[0011\]](#)
- [JP2002235236A \[0011\]](#)
- [JP2003284557A \[0011\]](#)
- [WO02062381A \[0011\]](#)
- [WO04029259A \[0011\]](#)
- [JP2005015346A \[0011\]](#)
- [EP1983047A \[0011\]](#)
- [JP2007205785A \[0193\]](#)

### Non-patent literature cited in the description

- IUPAC-IUB Communication on Biochemical Nomenclature Eur. J. Biochem., 1984, vol. 138, 9- [\[0020\]](#)
- Molecular Cloning Cold Spring Harbor Lab. Press 1989 0000 [\[0041\]](#)
- Idenshi Kenkyuho Zoku Seikagaku Jikken Kouza 1986 0000 vol. I, II, III, [\[0041\]](#) [\[0061\]](#)
- J. Am. Chem. Soc., 1967, vol. 89, 4801- [\[0042\]](#)
- J. AM. CHEM. SOC., 1969, vol. 91, 3350- [\[0042\]](#)
- Science, 1968, vol. 150, 178- [\[0042\]](#)
- Tetrahedron Lett., 1981, vol. 22, 1859- [\[0042\]](#)
- TETRAHEDRON LETT., 1983, vol. 24, 245- [\[0042\]](#)
- Nucleic Acids Res., 1981, vol. 9, 43- [\[0045\]](#)

- Proc. Natl. Acad. Sci., 1981, vol. 78, 6613- [\[0046\]](#)
- Science, 1983, vol. 222, 778- [\[0046\]](#)
- Science, 1985, vol. 230, 1350- [\[0052\]](#)
- Jikken Igaku, 1994, vol. 12, 635- [\[0052\]](#)
- **M. A. FROHMAN et al.** Proc. Natl. Acad. Sci., 1988, vol. 8, 8998- [\[0052\]](#)
- Proc. Natl. Acad. Sci., 1977, vol. 74, 5463- [\[0055\]](#)
- Methods in Enzymology, 1980, vol. 65, 499- [\[0055\]](#)
- Molecular Cloning 2d Ed Cold Spring Harbor Lab. Press 1989 0000 [\[0061\]](#)
- Science, 1984, vol. 224, 1431- [\[0075\]](#)
- Biochem. Biophys. Res. Comm., 1985, vol. 130, 692- [\[0075\]](#)
- Proc. Natl. Acad. Sci. USA, 1983, vol. 80, 5990- [\[0075\]](#)
- **OHNO et al.** Tanpaku Jikken Protocol 1 Functional Analysis Saibo Kogaku Bessatsu, Jikken Protocol Series, 1997, [\[0075\]](#)
- BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL Oxford University Press 1994 0000 [\[0075\]](#) [\[0089\]](#)
- **YOSHIDA et al.** Virology, 2003, vol. 316, 161-70 [\[0141\]](#)

## Patentkrav

1. Farmaceutisk sammensætning, der omfatter et rekombinant Autographa californica nukleopolyhedrosevirus (AcNPV) som et  
5 aktivt middel og eventuelt et farmaceutisk acceptabelt bæremateriale,  
hvor det rekombinante AcNPV indeholder en genstruktur med et fusionsgen af  
(A) et gen, der koder for en aminosyresekvens, der består af  
10 aminosyrerne 19-373 af SEQ ID NO: 70 (Plasmodium falciparum-circumsporozoitprotein; PfCSP), og  
(B) et gen, der koder for en aminosyresekvens, der består af aminosyrerne 21-512 af gp64-viruspartikelprotein (gp64), der kodes i AcNPV-genomet (GenBank-accessionsnr. L22858),  
15 under regulering af en dobbeltpromotor, der omfatter polyhedrinpromotoren og CAG-promotoren, der er koblet til hinanden (CAP).
2. Farmaceutisk sammensætning ifølge krav 1, som er en  
20 malarievaccine.

# DRAWINGS

Fig.1

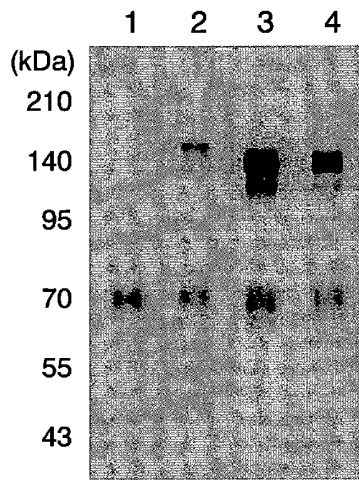


Fig.2(A)

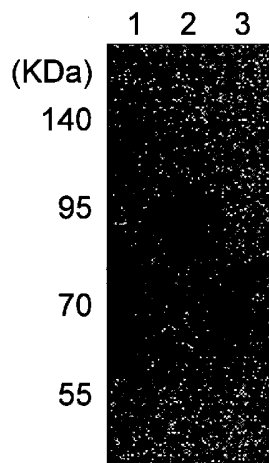


Fig.2(B)

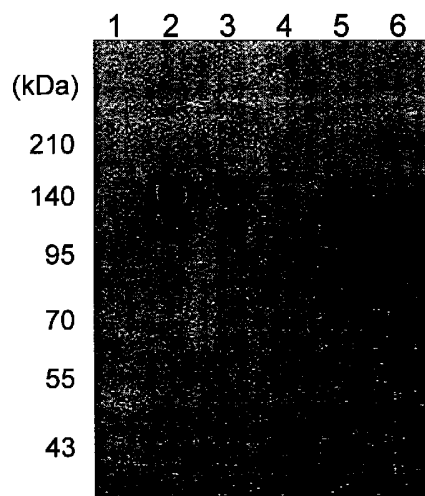


Fig.3

(A)

(B)



Fig.4(A)

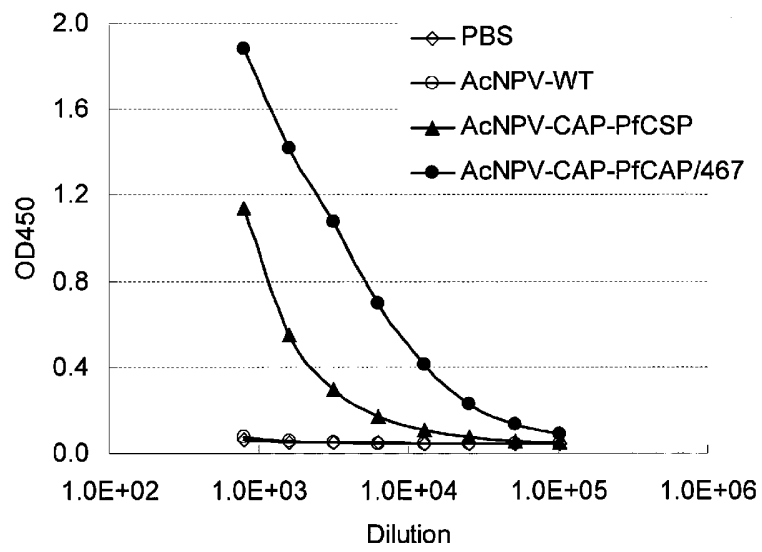


Fig.4(B)

