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(57) Abstract: The present invention provides caninized murine anti-human PD-1 antibodies that have specific sequences and a high binding affinity for canine PD-1. The invention also relates to use of these antibodies in the treatment of dogs.



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CANINIZED MURINE ANTIBODIES TO HUMAN PD-1

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application Serial No. 61/918,847, filed on December 20, 2013, the contents of which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to caninized murine antibodies to human PD-1 that have specific sequences and a high binding affinity for canine PD-1. The invention also relates to use of the antibodies of the present invention in the treatment of cancer in dogs.

BACKGROUND OF THE INVENTION

An immunoinhibitory receptor that is primarily expressed on activated T and B cells, Programmed Cell Death Receptor 1, also referred to as Programmed Death Receptor (PD-1), is a member of the immunoglobulin superfamily related to CD28 and CTLA-4. PD-1 and like family members are type I transmembrane glycoproteins containing an extracellular Ig Variable-type (V-type) domain that binds its ligands and a cytoplasmic tail that binds signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif).

PD-1 attenuates T-cell responses when bound to Programmed Cell Death Ligand 1, also referred to as Programmed Death Ligand 1 (PD-L1), and/or Programmed Cell Death Ligand 2, also referred to as Programmed Death Ligand 2 (PD-L2). The binding of either of these ligands to PD-1 negatively regulates antigen receptor signaling. Blocking the binding of PD-L1 to PD-1 enhances tumor-specific CD8⁺ T-cell immunity, while aiding the clearance of tumor cells by the immune system. The three-dimensional structure of murine PD-1, as well as the co-crystal structure of mouse PD-1 with human PD-L1 have been reported [Zhang *et al.*, *Immunity* 20: 337-347 (2004); Lin *et al.*, *Proc. Natl. Acad. Sci. USA* 105: 3011-3016 (2008)].

PD-L1 and PD-L2 are type I transmembrane ligands that contain both IgV- and IgC-like domains in the extracellular region along with short cytoplasmic regions with no known

signaling motifs. Both PD-L1 and PD-L2 are either constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as various tumor types. PD-L1 is not only expressed on B, T, myeloid and dendritic cells (DCs), but also on peripheral cells, such as microvascular endothelial cells and non-lymphoid organs *e.g.*, heart or lung. In contrast, PD-L2 is only found on macrophages and DCs. The expression pattern of PD-1 ligands suggests that PD-1 plays a role in maintaining peripheral tolerance and may further serve to regulate self-reactive T- and B-cell responses in the periphery.

In any case, it is now abundantly clear that PD-1 plays a critical role in at least certain human cancers, presumably by mediating immune evasion. Accordingly, PD-L1 has been shown to be expressed on a number of mouse and human tumors and is inducible by IFN *gamma* in the majority of PD-L1 negative tumor cell lines [Iwai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002); Strome *et al.*, *Cancer Res.*, 63: 6501-6505 (2003)]. Furthermore, the expression of PD-1 on tumor infiltrating lymphocytes and/or PD-L1 on tumor cells has been identified in a number of primary human tumor biopsies. Such tumor tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas, as well as tumors of the head and neck [Brown *et al.*, *J. Immunol.* 170: 1257-1266 (2003); Dong *et al.*, *Nat. Med.* 8: 793-800 (2002); Wintterle *et al.*, *Cancer Res.* 63: 7462-7467 (2003); Strome *et al.*, *Cancer Res.*, 63: 6501-6505 (2003); Thompson *et al.*, *Cancer Res.* 66: 3381-5 (2006); Thompson *et al.*, *Clin. Cancer Res.* 13: 1757-1761 (2007); Nomi *et al.*, *Clin. Cancer Res.* 13: 2151-2157. (2007)]. More strikingly, PD-ligand expression on tumor cells has been correlated to poor prognosis of human cancer patients across multiple tumor types [reviewed in Okazaki and Honjo, *Int. Immunol.* 19: 813-824 (2007)].

Moreover, Nomi *et al.* [*Clin. Cancer Res.* 13: 2151-2157 (2007)] demonstrated the therapeutic efficacy of blocking the binding of PD-L1 to PD-1 in a murine model of aggressive pancreatic cancer through administering either PD-1 or PD-L1 directed antibody. These antibodies effectively promoted tumor reactive CD8⁺ T cell infiltration into the tumor resulting in the up-regulation of anti-tumor effectors including IFN *gamma*, granzyme B, and perforin. Similarly, the use of antibodies to block the binding of PD-L1 and PD-1 significantly inhibited tumor growth in a model of mouse squamous cell carcinoma [Tsushima *et al.*, *Oral Oncol.* 42: 268-274 (2006)].

In other studies, transfection of a murine mastocytoma line with PD-L1 led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-L1 monoclonal antibody was added [Iwai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002)]. *In vivo*, blocking the PD1/PD-L1 interaction was shown to increase the efficacy of adoptive T cell transfer therapy in a mouse tumor model [Strome *et al.*, *Cancer Res.* 63: 6501-6505 (2003)]. Further evidence for the role of PD-1 in cancer treatment comes from experiments performed with PD-1 knockout mice in which PD-L1 expressing myeloma cells grew only in wild-type animals (resulting in tumor growth and associated animal death), but not in PD-1 deficient mice [Iwai Y. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002)]. More recently, antibodies against PD-1 (including humanized murine monoclonal antibodies against human PD-1) have shown at least initial success in cancer therapy in humans [*see e.g.*, US 8,354,509 B2, US 8,008,449 B2, and US 7,595,048 B2].

Anti-PD-1 antibodies may also be useful in chronic viral infection. Memory CD8⁺ T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber *et al.* [*Nature* 439: 682-687 (2006)] showed that mice infected with a laboratory strain of LCMV developed chronic infection resulted in high levels of virus in the blood and other tissues. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. Barber *et al.* found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-L1.

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

Summary of the Invention

The present invention relates to caninized murine anti-human PD-1 antibodies that have a high binding affinity to canine PD-1, as well as having the ability to block the binding of canine PD-1 to canine PD-L1. The present invention also relates to use of such antibodies in the treatment of disease such as cancer and/or those due to infections.

Accordingly, the present invention provides an isolated caninized antibody or antigen binding fragment thereof that specifically binds Programmed Death Receptor 1 (PD-1) comprising a canine IgG heavy chain and a canine *kappa* or *lambda* light chain. In particular embodiments of this type, the canine *kappa* or *lambda* light chain that comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and the canine IgG heavy chain comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3) obtained from a mammalian PD-1 antibody. Particular embodiments of the caninized antibodies and fragments thereof of the present invention bind canine PD-1 and/or block the binding of canine PD-1 to canine Programmed Death Ligand 1 (PD-L1).

The present invention also provides an isolated caninized antibody or an antigen binding fragment thereof that specifically binds Programmed Cell Death Receptor 1 (PD-1), wherein said antibody comprises a canine IgG heavy chain and a canine *kappa* light chain; wherein the canine *kappa* light chain comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and the canine IgG heavy chain comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3);

wherein the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 22, the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18;

wherein the antibody and the antigen binding fragment thereof bind canine PD-1 and block the binding of canine PD-1 to canine Programmed Cell Death Ligand 1 (PD-L1).

The present invention also provides an isolated nucleic acid that encodes the light chain of the caninized antibody according to any aspect, embodiment or example of the invention described herein.

The present invention also provides an isolated nucleic acid that encodes the heavy chain of the caninized antibody according to any aspect, embodiment or example of the invention described herein.

The present invention also provides an expression vector comprising the isolated nucleic acid according to any aspect, embodiment or example of the invention described herein.

The present invention also provides a host cell comprising one or more expression vectors according to any aspect, embodiment or example of the invention described herein.

The present invention also provides a pharmaceutical composition comprising the antibody or antigen binding fragment according to any aspect, embodiment or example of the invention described herein and a pharmaceutically acceptable carrier or diluent.

The present invention also provides a method of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition according to any aspect, embodiment or example of the invention described herein and a pharmaceutically acceptable carrier or diluent.

The present invention also provides the use of an effective amount of the antibody or antigen binding fragment according to any aspect, embodiment or example of the invention described herein in increasing the activity of an immune cell in a subject in need thereof.

The present invention also provides the use of the antibody or antigen binding fragment according to any aspect, embodiment or example of the invention described herein in the manufacture of a medicament for increasing the activity of an immune cell in a subject.

According to another aspect the present invention provides a method of producing a caninized antibody or antigen binding fragment thereof that specifically binds PD-1 comprising:

- a. culturing the host cell of the invention as described herein in culture medium under conditions wherein the nucleic acid is expressed, thereby producing a polypeptide comprising the light chain variable regions and a polypeptide comprising the heavy chain variable regions; and
- b. recovering the polypeptides from the host cell or culture medium

The present invention also provides a caninized antibody or antigen binding fragment thereof produced by performing the method according to the foregoing aspect of the invention.

In certain embodiments, canine light chain is a kappa chain. In particular embodiments of this type, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20. In related embodiments the CDRL1 comprises a conservatively modified variant of SEQ ID NO: 20. In other embodiments, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22. In related embodiments the CDRL2 comprises a conservatively modified variant of SEQ ID NO: 22. In still other embodiments the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24. In related embodiments the CDRL3 comprises a conservatively modified variant of SEQ ID NO: 24. In yet other embodiments the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14. In related embodiments the CDRH1 comprises a conservatively modified variant of SEQ ID NO: 14. In still other embodiments the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16. In related embodiments the CDRH2 comprises a conservatively modified variant of SEQ ID NO: 16. In yet other embodiments the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18. In related embodiments the CDRH3 comprises a conservatively modified variant of SEQ ID NO: 18.

In specific embodiments the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20 or a conservatively modified variant of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22 or a conservatively modified variant of SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24 or a conservatively modified variant of SEQ ID NO: 24.

In other specific embodiments the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14 or a conservatively modified variant of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16 or a conservatively modified variant of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18 or a conservatively modified variant of SEQ ID NO: 18.

In a more specific embodiment the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20 or a conservatively modified variant of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22 or a conservatively modified variant of SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24 or a conservatively modified variant of SEQ ID NO: 24, and the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14 or a conservatively modified variant of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16 or a conservatively modified variant of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18 or a conservatively modified variant of SEQ ID NO: 18.

In an even more specific embodiment the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18.

For embodiments of the present invention, the IgG heavy chain comprises an amino acid sequence of SEQ ID NO: 26. In related embodiments the IgG heavy chain comprises a conservatively modified variant of SEQ ID NO: 26. In other embodiments the IgG heavy chain comprises an amino acid sequence of SEQ ID NO: 28. In related embodiments the IgG

heavy chain comprises a conservatively modified variant of SEQ ID NO: 28. In still other embodiments the IgG heavy chain comprises an amino acid sequence of SEQ ID NO: 30. In related embodiments the IgG heavy chain comprises a conservatively modified variant of SEQ ID NO: 30.

In certain embodiments the *kappa* light chain comprises an amino acid sequence of SEQ ID NO: 32. In related embodiments, the *kappa* light chain comprises conservatively modified variant of SEQ ID NO: 32. In particular embodiments the *kappa* light chain comprises an amino acid sequence of SEQ ID NO: 34. In related embodiments, the *kappa* light chain comprises conservatively modified variant of SEQ ID NO: 34.

In a more particular embodiment, an isolated caninized antibody comprises the amino acid sequence of SEQ ID NO: 28 and of SEQ ID NO: 34. In related embodiments the isolated caninized antibody comprises a conservatively modified variant of SEQ ID NO: 28 and a conservatively modified variant of SEQ ID NO: 34. In still other related embodiment the isolated caninized antibody comprises the amino acid sequence of SEQ ID NO: 28 and a conservatively modified variant of SEQ ID NO: 34. In yet other related embodiment the isolated caninized antibody comprises a conservatively modified variant of SEQ ID NO: 28 and the amino acid sequence of SEQ ID NO: 34.

The present invention further provides isolated nucleic acids that encode any one of the light chains of the caninized antibody of the present invention. Similarly, the present invention further provides isolated nucleic acids that encode any one of the heavy chains of the caninized antibody of the present invention. The present invention further provides expression vectors that comprise one or more of the isolated nucleic acids of the present invention. The present invention further provides host cells that comprise one or more expression vectors of the present invention.

In particular embodiments, the antibody is a recombinant antibody or an antigen binding fragment thereof. In related embodiments, the variable heavy chain domain and variable light chain domain are connected by a flexible linker to form a single-chain antibody.

In particular embodiments, the antibody or antigen binding fragment is a Fab fragment.

In other embodiments, the antibody or antigen binding fragment is a Fab' fragment. In other embodiments, the antibody or antigen binding fragment is a (Fab')₂ fragment. In still other embodiments, the antibody or antigen binding fragment is a diabody. In particular embodiments, the antibody or antigen binding fragment is a domain antibody. In particular embodiments, the antibody or antigen binding fragment is a camelized single domain antibody.

In particular embodiments, the caninized murine anti-human PD-1 antibody or antigen binding fragment increases the immune response of the canine subject being treated.

The present invention further provides isolated nucleic acids that encode the caninized murine anti-human PD-1 antibodies or antigen binding fragments as disclosed herein. In related embodiments such antibodies or antigen binding fragments can be used for the preparation of a medicament to treat cancer in a canine subject. Alternatively, or in conjunction, the present invention provides for the use of any of the antibodies or antibody fragments of the present invention for diagnostic use. In yet additional embodiments, a kit is provided comprising any of the caninized antibodies or antigen binding fragments disclosed herein.

In yet additional embodiments, an expression vector is provided comprising an isolated nucleic acid encoding any of the caninized murine anti-human PD-1 antibodies or antigen binding fragments of the invention. The invention also relates to a host cell comprising any of the expression vectors described herein. In particular embodiments, these nucleic acids, expression vectors or polypeptides of the invention are useful in methods of making an antibody.

The present invention further includes pharmaceutical compositions comprising an antibody or antigen binding fragment thereof together with a pharmaceutically acceptable carrier or diluent. In addition, the present invention provides methods of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of such pharmaceutical compositions. In certain embodiments the method is used for the treatment of cancer. In other embodiments, the method is used in the treatment of an infection or infectious disease. In still other embodiments, a caninized antibody of the present invention or antigen binding fragment thereof is used as a vaccine adjuvant.

These and other aspects of the present invention will be better appreciated by reference to the following Brief Description of the Drawings and the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the reactivity of murine anti-human PD-1 monoclonal antibody 08A [mAb 08A; as first described in US 8,354,509 B2 in relation to human PD-1] against the His-tagged extracellular domain of canine PD-1.

Figure 2 shows the reactivity of murine anti-human PD-1 monoclonal antibody 08A (see above) against canine PD-1 proteins expressed on CHO cells using CELISA. Murine anti-human PD-1 monoclonal antibody 08A and its caninized variants were found to react with canine PD-1 in a dose dependent manner.

Figure 3 depicts the ligand blockade by murine and caninized monoclonal antibodies. Murine anti-human PD-1 monoclonal antibody 08A (see above) and its caninized variants blocked the binding of canine PD-L1 to PD-1 expressed on CHO cell surface.

Figure 4 provides the alignment of canine IgGB constant heavy chains (CHs) lacking ADCC function. The canine wild type IgB [cIgGB wt], Canine IgGB(+)A-hinge [cIgGB(+) A-hinge], Canine IgGB(+) D-hinge [cIgGB(+) D-hinge], and Canine IgGB (-)ADCC [cIgGB(-) ADCC] are depicted. The (+) A-hinge is the replacement with IgG-A hinge *plus* a lysine and asparagine amino acid replacement as shown; the (+) D-hinge is the replacement with IgG-D hinge *plus* a lysine and the asparagine amino acid replacement as shown. The (-)ADCC is the lysine and asparagine amino acid replacement.

DETAILED DESCRIPTION**Abbreviations**

Throughout the detailed description and examples of the invention the following abbreviations will be used:

ADCC	Antibody-dependent cellular cytotoxicity
CDC	Complement-dependent cytotoxicity
CDR	Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system
CHO	Chinese hamster ovary
EC50	concentration resulting in 50% efficacy or binding
ELISA	Enzyme-linked immunosorbant assay
FR	Antibody framework region: the immunoglobulin variable regions excluding the CDR regions.
HRP	Horseradish peroxidase
IFN	interferon
IC50	concentration resulting in 50% inhibition
IgG	Immunoglobulin G
Kabat	An immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat [<i>Sequences of Proteins of Immunological Interest</i> , 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]
mAb	Monoclonal antibody (also Mab or MAb)
MES	2-(N-morpholino)ethanesulfonic acid
MOA	Mechanism of action
NHS	Normal human serum
PCR	Polymerase chain reaction
PK	Pharmacokinetics
SEB	Staphylococcus Enterotoxin B
TT	Tetanus toxoid
V region	The segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.
VH	Immunoglobulin heavy chain variable region

VK

Immunoglobulin *kappa* light chain variable region**DEFINITIONS**

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

"Activation" as it applies to cells or to receptors refers to the activation or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. "Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compounds derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors.

"Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton. "Activity" can also mean specific activity, e.g., [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], concentration in a biological compartment, or the like. "Activity" may refer to modulation of components of the innate or the adaptive immune systems.

"Administration" and "treatment," as it applies to an animal, e.g., a canine experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal e.g., a canine

subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (e.g., canine, feline, or human) and most preferably a canine.

"Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies or antigen binding fragments of the present invention, internally or externally to a canine subject or patient having one or more disease symptoms, or being suspected of having a disease, for which the agent has therapeutic activity. Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the patient (e.g., canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veterinarians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (e.g., a treatment method or article of manufacture) may not be effective in alleviating the target disease symptom(s) in every subject, it should alleviate the target disease symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the χ^2 -test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a human, veterinary (e.g., canine) or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a human, veterinary (e.g., canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies or antigen binding fragments of the present invention to a canine or other animal subject, a cell, tissue, physiological compartment, or physiological fluid.

Canine PD-1 has been found to comprise the amino acid sequence of SEQ ID NO: 2. In a specific embodiment canine PD-1 is encoded by a nucleic acid that comprises the nucleotide sequence of SEQ ID NO: 1. Canine PD-1 sequences may differ by having, for example, conserved variations in non-conserved regions, but the canine PD-1 will have substantially the same biological function as the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2. For example, a biological function of PD-1 is to attenuate T-cell responses when bound to PD-L1 and/or PD-L2. That is, PD-1 may be considered a negative regulator. Notably, the cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif). In addition, a biological function of canine PD-1 may be having, for example, an epitope in the extracellular domain that is specifically bound by an antibody of the instant disclosure.

Canine PD-L1 has been found to comprise the amino acid sequence of SEQ ID NO: 8. In a specific embodiment canine PD-L1 is encoded by a nucleotide sequence comprising SEQ ID NO: 7. Canine PD-L1 sequences may differ by having, for example, conserved variations in non-conserved regions, but the canine PD-L1 will have substantially the same biological function as the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8. For example, one biological function of PD-L1 is to attenuate T-cell responses when bound to PD-1.

A particular canine PD-1 or PD-L1 amino acid sequence respectively, will generally be at least 90% identical to the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain cases, a canine PD-1 or PD-L1 respectively, may be at least 95%, or even at least 96%, 97%, 98% or 99% identical to the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain embodiments, a canine PD-1 or a PD-L1 amino acid sequence will display no more than 10 amino acid differences from the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain embodiments, the canine PD-1 or the PD-L1 amino acid sequence respectively, may display no more than 5, or even no more than 4, 3, 2, or 1

amino acid difference from the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. Percent identity can be determined as described herein below.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the mammalian body (*e.g.*, canine body) of cancerous cells, cells or tissues infected with pathogens, or invading pathogens.

Caninized Anti-Human PD-1 Antibodies

The present invention provides isolated caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof that bind canine PD-1 and uses of such antibodies or fragments.

As used herein, a caninized murine anti-human PD-1 antibody refers to a caninized antibody that specifically binds to mammalian PD-1. An antibody that specifically binds to mammalian PD-1, and in particular canine PD-1, is an antibody that exhibits preferential binding to mammalian PD-1 as compared to other antigens, but this specificity does not require absolute binding specificity. A caninized murine anti-human PD-1 antibody is considered "specific" for canine PD-1 if its binding is determinative of the presence of canine PD-1 in a biological sample obtained from a canine, or if it is capable of altering the activity of canine PD-1 without unduly interfering with the activity of other canine proteins in a canine sample, *e.g.* without producing undesired results such as false positives in a diagnostic context or side effects in a therapeutic context. The degree of specificity necessary for a caninized murine anti-human PD-1 antibody may depend on the intended use of the antibody, and at any rate is defined by its suitability for use for an intended purpose. The antibody, or binding compound derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, preferably at least ten-times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with any other canine antigen.

As used herein, an antibody is said to bind specifically to a polypeptide comprising a given sequence (in this case canine PD-1) if it binds to polypeptides comprising the sequence of canine PD-1, but does not bind to other canine proteins lacking the amino acid sequence of canine PD-1. For example, an antibody that specifically binds to a polypeptide comprising canine PD-1 may bind to a FLAG[®]-tagged form of canine PD-1, but will not bind to other FLAG[®]-tagged canine proteins.

As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, *i.e.* antibody fragments that retain the ability to bind specifically to the antigen (*e.g.*, canine PD-1) bound by the full-length antibody, *e.g.* fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, *e.g.*, sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

Typically, a caninized antibody or antigen binding fragment thereof of the invention retains at least 10% of its canine PD-1 binding activity (when compared to the corresponding parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the canine PD-1 binding affinity as the parental antibody. It is also intended that an antibody or antigen binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

"Isolated antibody" refers to the purification status and in such context means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

The variable regions of each light/heavy chain pair form the antigen binding site of the antibody. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). The CDRs are usually flanked by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of *Sequences of Proteins of Immunological Interest*, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978); Kabat, *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987) or Chothia, *et al.*, *Nature* 342:878-883 (1989)].

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 in the heavy chain variable domain). [See Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), defining the CDR regions of an antibody by sequence; *see also* Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987) defining the CDR regions of an antibody by structure]. As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

As used herein, the term "canine" includes all domestic dogs, *Canis lupus familiaris* or *Canis familiaris*, unless otherwise indicated.

As used herein the term "canine frame" refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in the majority of embodiments the

amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse antibody) in both chains. Optionally the heavy and/or light chains of the canine antibody may contain some foreign non-CDR residues, *e.g.*, so as to preserve the conformation of the foreign CDRs within the canine antibody, and/or to modify the Fc function, as discussed below.

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa*.

Besides binding and activating of canine immune cells, a canine or caninized antibody against PD-1 optimally has two attributes:

1. Lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
2. be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has high level of ADCC activity. On the other hand, IgG-A binds weakly to protein A, but displays undesirable ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D display no ADCC activity. (IgG-C has considerable ADCC activity). The present invention overcomes this difficulty by providing mutant canine IgG-B antibodies specific to PD-1; such antibodies lack effector functions such as ADCC and can be easily purified using industry standard protein A chromatography.

As used herein, the term "caninized antibody" refers to an antibody that comprises the three heavy chain CDRs and the three light chain CDRS from a murine anti-human PD-1 antibody together with a canine frame or a modified canine frame. A modified canine frame comprises one or more amino acids changes as exemplified herein that further optimize the effectiveness of the caninized antibody, *e.g.*, to increase its binding to canine PD-1 and/or its ability to block the binding of canine PD-1 to canine PD-L1.

"Homology" refers to sequence similarity between two polynucleotide sequences or between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared $\times 100$. For example, if 6 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The phrase "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to use promoters, polyadenylation signals, and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked"

means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. It should also be readily understood that when a nucleic acid sequence is provided herein, it may include a stop codon. However, as stop codons are interchangeable the inclusion of a specific stop codon in a sequence should not be viewed as a necessary portion of that sequence.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that not all progeny will have precisely identical DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

As used herein, "germline sequence" refers to a sequence of unrearranged immunoglobulin DNA sequences. Any suitable source of unrearranged immunoglobulin sequences may be used. Human germline sequences may be obtained, for example, from JOINSOLVER[®] germline databases on the website for the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the United States National Institutes of Health. Mouse germline sequences may be obtained, for example, as described in Giudicelli *et al.* [*Nucleic Acids Res.* 33:D256-D261 (2005)].

Properties of the Exemplary Caninized Murine Anti-human PD-1 Antibodies

The present invention provides isolated caninized murine anti-human PD-1 antibodies and methods of use of the antibodies or antigen binding fragments thereof in the treatment of disease *e.g.*, the treatment of cancer in canines. Examples of caninized murine anti-human PD-1 antibodies that bind canine PD-1 include, but are not limited to: antibodies that comprise canine IgG-A, IgG-B, and IgG-D heavy chains and/or canine *kappa* light chains together with murine anti-human PD-1 CDRs. Accordingly, the present invention provides isolated

caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof that bind to canine PD-1 and block the binding of canine PD-1 to canine PD-L1.

The isolated antibody or antigen binding fragment thereof that binds canine PD-1 can comprise one, two, three, four, five, or six of the complementarity determining regions (CDRs) of the murine anti-human antibody as described herein. The one, two, three, four, five, or six CDRs may be independently selected from the CDR sequences of those provided in Table 2 in the Examples below. In certain embodiments, one, two or three CDRs are selected from the V_L CDRs (amino acid SEQ ID NOs: 20, 22, and/or 24) and/or one, two or three CDRs selected from the V_H CDRs (SEQ ID NOs: 14, 16, and/or 18), and/or conservatively modified variants of the one, two or three of these V_L CDRs and/or conservatively modified variants of the one, two or three of these V_H CDRs.

In a further embodiment, the isolated antibody or antigen-binding fragment thereof that binds canine PD-1 comprises a canine antibody *kappa* light chain comprising a murine light chain CDR-1, CDR-2 and/or CDR-3 and a canine antibody heavy chain IgG comprising a murine heavy chain CDR-1, CDR-2 and/or CDR-3.

In other embodiments, the invention provides antibodies or antigen binding fragments thereof that specifically binds PD-1 and have canine antibody *kappa* light chains comprising CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID NOs: 20, 22, and/or 24 and canine antibody heavy chain IgG with CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID NOs: 14, 16, and/or 18, while still exhibiting the desired binding and functional properties. In another embodiment the antibody or antigen binding fragment of the present invention comprises a canine frame comprising of a combination of IgG heavy chain sequence (comprising an amino acid sequence of SEQ ID NO: 26, 28, or 30 with and without signal sequence) with a *kappa* light chain (comprising an amino acid sequence of SEQ ID NO: 32, or 34 with and without signal sequence) having up to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more conservative or non-conservative amino acid substitutions, while still exhibiting the desired binding and functional properties. In a particular embodiment of this type, the number of conservative amino acid substitutions is between 0 to 5 for the IgG heavy chain and 0 to 5 for the *kappa* light chain.

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity [*see, e.g., Watson et al., Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.; 1987)]. In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Various embodiments of the antibody or antigen binding fragment of the present invention comprise polypeptide chains with the sequences disclosed herein, *e.g.*, SEQ ID NOs: 26, 28, 30, 32, and/or 34, or polypeptide chains comprising up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more conservative amino acid substitutions. Exemplary conservative substitutions are set forth in Table I.

TABLE I.**Exemplary Conservative Amino Acid Substitutions**

Original residue	Conservative substitution
Ala (A)	Gly; Ser;
Arg (R)	Lys; His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala

Original residue	Conservative substitution
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants," as used herein, refers to antibodies or fragments in which one or more amino acid residues have been changed without altering a desired property, such as an antigen affinity and/or specificity. Such variants include, but are not limited to, replacement of an amino acid with one having similar properties, such as the conservative amino acid substitutions of Table I.

Nucleic Acids

The present invention further comprises the nucleic acids encoding the immunoglobulin chains of caninized murine anti-human PD-1 antibodies and antigen binding fragments thereof disclosed herein. For example, the present invention includes the nucleic acids listed in Tables 2 and 3 and the Sequence Listing Table below.

Also included in the present invention are nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the amino acid sequences of the antibodies provided herein when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. The present invention further provides nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences when the comparison is performed with a BLAST algorithm, wherein the parameters of the algorithm are selected to give the largest match between the respective

sequences over the entire length of the respective reference sequences, are also included in the present invention.

Sequence identity refers to the degree to which the amino acids of two polypeptides are the same at equivalent positions when the two sequences are optimally aligned. Sequence similarity includes identical residues and nonidentical, biochemically related amino acids. Biochemically related amino acids that share similar properties and may be interchangeable are discussed above.

The following references relate to BLAST algorithms often used for sequence analysis:

BLAST ALGORITHMS: Altschul, S.F., *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish, W., *et al.*, *Nature Genet.* 3:266-272 (1993); Madden, T.L., *et al.*, *Meth. Enzymol.* 266:131-141(1996); Altschul, S.F., *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang, J., *et al.*, *Genome Res.* 7:649-656 (1997); Wootton, J.C., *et al.*, *Comput. Chem.* 17:149-163 (1993); Hancock, J.M. *et al.*, *Comput. Appl. Biosci.* 10:67-70 (1994); ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, (1978); *Natl. Biomed. Res. Found.*, Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3." (1978), M.O. Dayhoff (ed.), pp. 353-358 (1978), *Natl. Biomed. Res. Found.*, Washington, DC; Altschul, S.F., *J. Mol. Biol.* 219:555-565 (1991); States, D.J., *et al.*, *Methods* 3:66-70(1991); Henikoff, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992); Altschul, S.F., *et al.*, *J. Mol. Evol.* 36:290-300 (1993); ALIGNMENT STATISTICS: Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:2264-2268 (1990); Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993); Dembo, A., *et al.*, *Ann. Prob.* 22:2022-2039 (1994); and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in *Theoretical and Computational Methods in Genome Research* (S. Suhai, ed.), pp. 1-14, Plenum, New York (1997).

This present invention also provides expression vectors comprising the isolated nucleic acids of the invention, wherein the nucleic acid is operably linked to control sequences that are recognized by a host cell when the host cell is transfected with the vector. Also provided are host cells comprising an expression vector of the present invention and methods for producing the antibody or antigen binding fragment thereof disclosed herein comprising culturing a host

cell harboring an expression vector encoding the antibody or antigen binding fragment in culture medium, and isolating the antigen or antigen binding fragment thereof from the host cell or culture medium.

Epitope Binding and Binding Affinity

The present invention further provides antibodies or antigen binding fragments thereof that bind to the same epitope on canine PD-1 as the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and/or of SEQ ID NO: 32, or the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and/or of SEQ ID NO: 34. The caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof are capable of inhibiting the binding of canine PD-1 to canine PD-L1.

The caninized murine anti-human PD-1 antibody can be produced recombinantly as described below in the examples. Mammalian cell lines available as hosts for expression of the antibodies or fragments disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion or fragment thereof, the light chain and/or antigen-binding fragment thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the

glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In general, glycoproteins produced in a particular cell line or transgenic animal will have a glycosylation pattern that is characteristic for glycoproteins produced in the cell line or transgenic animal. Therefore, the particular glycosylation pattern of an antibody will depend on the particular cell line or transgenic animal used to produce the antibody. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein, comprise the instant invention, independent of the glycosylation pattern that the antibodies may have. Similarly, in particular embodiments, antibodies with a glycosylation pattern comprising only non-fucosylated *N*-glycans may be advantageous, because these antibodies have been shown to typically exhibit more potent efficacy than their fucosylated counterparts both *in vitro* and *in vivo* [See for example, Shinkawa *et al.*, *J. Biol. Chem.* 278: 3466-3473 (2003); U.S. Patent Nos. 6,946,292 and 7,214,775].

The present invention further includes antibody fragments of the caninized murine anti-human PD-1 antibodies disclosed herein. The antibody fragments include F(ab)₂ fragments, which may be produced by enzymatic cleavage of an IgG by, for example, pepsin. Fab fragments may be produced by, for example, reduction of F(ab)₂ with dithiothreitol or mercaptoethylamine. A Fab fragment is a V_L-C_L chain appended to a V_H-C_{H1} chain by a disulfide bridge. A F(ab)₂ fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an F(ab)₂ molecule includes a portion of the F_c region between which disulfide bridges are located. An F_V fragment is a V_L or V_H region.

In one embodiment, the antibody or antigen binding fragment comprises a heavy chain constant region, *e.g.*, a canine constant region, such as IgG-A, IgG-B, IgG-C and IgG-D canine heavy chain constant region or a variant thereof. In another embodiment, the antibody or antigen binding fragment comprises a light chain constant region, *e.g.*, a canine light chain constant region, such as *lambda* or *kappa* canine light chain region or variant thereof. By way

of example, and not limitation the canine heavy chain constant region can be from IgG-D and the canine light chain constant region can be from *kappa*.

Antibody Engineering

The caninized murine anti-human PD-1 antibodies of the present invention have been engineered to include modifications to framework residues within the variable domains of a parental (i.e., canine) monoclonal antibody, *e.g.* to improve the properties of the antibody.

Experimental and diagnostic uses

Caninized murine anti-human PD-1 antibodies or antigen-binding fragments thereof of the present invention may also be useful in diagnostic assays for canine PD-1 protein, *e.g.*, detecting its expression in specific tumor cells, tissues, or serum. Such diagnostic methods may be useful in various disease diagnoses, particularly certain cancers in canines.

For example, such a method comprises the following steps:

- (a) coat a substrate (*e.g.*, surface of a microtiter plate well, *e.g.*, a plastic plate) with caninized murine anti-human PD-1 antibody or an antigen-binding fragment thereof;
- (b) apply a sample to be tested for the presence of canine PD-1 to the substrate;
- (c) wash the plate, so that unbound material in the sample is removed;
- (d) apply detectably labeled antibodies (*e.g.*, enzyme-linked antibodies) which are also specific to the PD-1 antigen;
- (e) wash the substrate, so that the unbound, labeled antibodies are removed;
- (f) if the labeled antibodies are enzyme linked, apply a chemical which is converted by the enzyme into a fluorescent signal; and
- (g) detect the presence of the labeled antibody.

In a further embodiment, the labeled antibody is labeled with peroxidase which react with ABTS [*e.g.*, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] or 3,3',5,5'-Tetramethylbenzidine to produce a color change which is detectable. Alternatively, the labeled antibody is labeled with a detectable radioisotope (*e.g.*, ³H) which can be detected by scintillation counter in the presence of a scintillant. Caninized murine anti-human PD-1 antibodies of the invention may be used in a Western blot or immuno protein blot procedure.

Such a procedure forms part of the present invention and includes for example:

(i) contacting a membrane or other solid substrate to be tested for the presence of bound canine PD-1 or a fragment thereof with a caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof of the present invention. Such a membrane may take the form of a nitrocellulose or vinyl-based [*e.g.*, polyvinylidene fluoride (PVDF)] membrane to which the proteins to be tested for the presence of canine PD-1 in a non-denaturing PAGE (polyacrylamide gel electrophoresis) gel or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gel have been transferred (*e.g.*, following electrophoretic separation in the gel). Before contact of membrane with the caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof, the membrane is optionally blocked, *e.g.*, with non-fat dry milk or the like so as to bind non-specific protein binding sites on the membrane.

(ii) washing the membrane one or more times to remove unbound caninized murine anti-human PD-1 antibody or an antigen-binding fragment thereof and other unbound substances; and

(iii) detecting the bound caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof.

Detection of the bound antibody or antigen-binding fragment may be by binding the antibody or antigen-binding fragment with a secondary antibody (an anti-immunoglobulin antibody) which is detectably labeled and, then, detecting the presence of the secondary antibody.

The caninized murine anti-human PD-1 antibodies and antigen-binding fragments thereof disclosed herein may also be used for immunohistochemistry. Such a method forms part of the present invention and comprises, *e.g.*, (1) contacting a cell to be tested for the presence of canine PD-1 with a caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof of the present invention; and (2) detecting the antibody or fragment on or in the cell.

If the antibody or antigen-binding fragment itself is detectably labeled, it can be detected directly. Alternatively, the antibody or antigen-binding fragment may be bound by a detectably labeled secondary antibody which is detected.

Certain caninized murine anti-human PD-1 antibodies and antigen-binding fragments thereof disclosed herein may also be used for *in vivo* tumor imaging. Such a method may include

injection of a radiolabeled caninized murine anti-human PD-1 antibodies or antigen-binding fragment thereof into the body of a canine to be tested for the presence of a tumor associated with canine PD-1 expression followed by nuclear imaging of the body of the patient to detect the presence of the labeled antibody or antigen-binding fragment *e.g.*, at loci comprising a high concentration of the antibody or antigen-binding fragment which are bound to the tumor.

Imaging techniques include SPECT imaging (single photon emission computed tomography) or PET imaging (positron emission tomography). Labels include *e.g.*, iodine-123 (^{123}I) and technetium-99m ($^{99\text{m}}\text{Tc}$), *e.g.*, in conjunction with SPECT imaging or ^{11}C , ^{13}N , ^{15}O or ^{18}F , *e.g.*, in conjunction with PET imaging or Indium-111 [See *e.g.*, Gordon *et al.*, *International Rev. Neurobiol.* 67:385-440 (2005)].

Pharmaceutical Compositions and Administration

To prepare pharmaceutical or sterile compositions of the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof is admixed with a pharmaceutically acceptable carrier or excipient. [See, *e.g.*, *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984)].

Formulations of therapeutic and diagnostic agents may be prepared by mixing with acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions or suspensions [see, *e.g.*, Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY]. In one embodiment, anti-PD-1 antibodies of the present invention are diluted to an appropriate concentration in a sodium acetate solution pH 5-6, and NaCl or sucrose is added for tonicity. Additional agents, such as polysorbate 20 or polysorbate 80, may be added to enhance stability.

Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with another agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD₅₀/ ED₅₀). In particular aspects, antibodies exhibiting high therapeutic indices are desirable. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in canines. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The mode of administration can vary. Suitable routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial.

In particular embodiments, the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof can be administered by an invasive route such as by injection. In further embodiments of the invention, a caninized murine anti-human PD-1 antibody or antigen binding fragment thereof, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, intratumorally, or by inhalation, aerosol delivery. Administration by non-invasive routes (*e.g.*, orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle, including, *e.g.*, a prefilled syringe or autoinjector. The pharmaceutical compositions disclosed herein may also be administered with a needleless hypodermic injection device; such as the devices disclosed in U.S. Patent Nos. 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

The pharmaceutical compositions disclosed herein may also be administered by infusion. Examples of well-known implants and modules for administering pharmaceutical compositions include: U.S. Patent No. 4,487,603, which discloses an implantable micro-

infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

Alternately, one may administer the caninized murine anti-human PD-1 antibody in a local rather than systemic manner, for example, via injection of the antibody directly into an arthritic joint or pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the caninized murine anti-human PD-1 antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, arthritic joint or pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibody, the level of symptoms, the immunogenicity of the therapeutic antibody, and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibody to effect improvement in the target disease state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibody and the severity of the condition being treated. Guidance in selecting appropriate doses of therapeutic antibodies is available [*see, e.g., Wawrzynczak Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK (1996); Kresina (ed.) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY (1991); Bach (ed.) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY (1993); Baert, *et al. New Engl. J. Med.* 348:601-608 (2003); Milgrom *et al. New Engl. J. Med.* 341:1966-1973 (1999); Slamon *et al. New Engl. J. Med.* 344:783-792 (2001); Beniaminovitz *et al. New Engl. J. Med.* 342:613-619 (2000); Ghosh *et al. New Engl. J. Med.* 348:24-32 (2003); Lipsky *et al. New Engl. J. Med.* 343:1594-1602 (2000)].

Determination of the appropriate dose is made by the veterinarian, *e.g.*, using parameters or factors known or suspected in the art to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

Antibodies or antigen binding fragments thereof disclosed herein may be provided by continuous infusion, or by doses administered, *e.g.*, daily, 1-7 times per week, weekly, bi-weekly, monthly, bimonthly, quarterly, semiannually, annually etc. Doses may be provided, *e.g.*, intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A total weekly dose is generally at least 0.05 µg/kg body weight, more generally at least 0.2 µg/kg, 0.5 µg/kg, 1 µg/kg, 10 µg/kg, 100 µg/kg, 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/ml, 10 mg/kg, 25 mg/kg, 50 mg/kg or more [*see, e.g.*, Yang, *et al. New Engl. J. Med.* 349:427-434 (2003); Herold, *et al. New Engl. J. Med.* 346:1692-1698 (2002); Liu, *et al. J. Neurol. Neurosurg. Psych.* 67:451-456 (1999); Portielji, *et al. Cancer Immunol. Immunother.* 52:133-144 (2003)]. Doses may also be provided to achieve a pre-determined target concentration of the caninized murine anti-human PD-1 antibody in the subject's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 µg/ml or more. In other embodiments, a caninized murine anti-human PD-1 antibody of the present invention is administered subcutaneously or intravenously, on a weekly, biweekly, "every 4 weeks," monthly, bimonthly, or quarterly basis at 10, 20, 50, 80, 100, 200, 500, 1000 or 2500 mg/subject.

As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with a disorder and/or a reduction in the severity of the symptoms of such disorder. The terms further include ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with a disorder, disease or symptom, or with the potential to develop such a disorder, disease or symptom.

As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of the caninized murine anti-human PD-1 antibody

or antigen binding fragment thereof of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, is effective to cause a measurable improvement in one or more symptoms of a disease or condition or the progression of such disease or condition. A therapeutically effective dose further refers to that amount of the binding compound sufficient to result in at least partial amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. An effective amount of a therapeutic will result in an improvement of a diagnostic measure or parameter by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. An effective amount can also result in an improvement in a subjective measure in cases where subjective measures are used to assess disease severity.

Other Combination Therapies

As previously described, the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof may be coadministered with one or other more therapeutic agents (such as a chemotherapeutic agent). The antibody may be linked to the agent (as an immunocomplex) or can be administered separately from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies.

Kits

Further provided are kits comprising one or more components that include, but are not limited to, an antibody or antigen binding fragment, as discussed herein, which specifically binds PD-1 (*e.g.*, a caninized murine anti-human PD-1 antibody or antigen binding fragment thereof of the present invention) in association with one or more additional components including, but not limited to a pharmaceutically acceptable carrier and/or a chemotherapeutic agent, as discussed herein. The binding composition and/or the chemotherapeutic agent can be formulated as a

pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

In one embodiment, the kit includes a binding composition of the invention (the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and of SEQ ID NO: 32 or 34, or a pharmaceutical composition thereof in one container (*e.g.*, in a sterile glass or plastic vial) and a pharmaceutical composition thereof and/or a chemotherapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial).

In another embodiment, the kit comprises a combination of the invention, including a binding composition component (*e.g.*, the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and of SEQ ID NO: 32 or 34) along with a pharmaceutically acceptable carrier, optionally in combination with one or more therapeutic agent component formulated together, optionally, in a pharmaceutical composition, in a single, common container.

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above. The kit can also include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids pet owners and veterinarians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdose, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

As a matter of convenience, an antibody or specific binding agent disclosed herein can be provided in a kit, *i.e.*, a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic or detection assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (*e.g.*, a substrate precursor which provides the detectable chromophore or fluorophore). In addition,

other additives may be included such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

EXAMPLES

EXAMPLE 1

CANINE PD-1 AND PD-L1

Identification and Cloning of Canine PD-1:

A nucleic acid encoding a full length canine PD-1 (cPD-1) was identified through a search of the NCBI gene bank data bases (accession number XM_543338.4, SEQ ID NO: 1). The translated amino acid sequence SEQ ID NO: 2 (accession number XP-543338.3) corresponds to putative canine PD-1 protein which was further identified through searching the gene bank (NCBI) protein databases and aligning the identified amino acid sequence with murine, feline, and human PD-1 amino acid sequences. The DNA sequence corresponding to the full length canine PD-1 gene that was codon optimized for CHO cells was synthesized and cloned into a plasmid designated p96793. Comparison of DNA and protein sequences of predicted canine PD-1 with known PD-1 DNA and protein sequences led to the identification of the DNA sequences encoding the extra-cellular domain (ECD) of canine PD-1 (SEQ ID NO: 3) and the amino acid sequence of the ECD of canine PD-1 (SEQ ID NO: 4).

A DNA sequence encoding the ECD of canine PD-1 in addition to a GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2726. A nucleic acid sequence (SEQ ID NO: 5) corresponding to the canine PD-1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene was chemically synthesized and cloned into a plasmid designated LPD2727. Canine PD-1 ECD and the Fc part of human IgG1 Fc comprises the amino acid sequence of SEQ ID NO: 6.

Identification and Cloning of Canine PD-L1:

A nucleic acid encoding a full length canine PD-L1 was identified through a search of the NCBI gene bank data bases (accession number XM_541302.4; SEQ ID NO: 7). The translated amino acid sequence (accession number XP-541302.4; SEQ ID NO: 8) corresponding to the putative canine PD-L1 protein was identified by searching the gene bank (NCBI) protein databases and alignment of the identified sequence with known PD-L1 mouse and human sequences.

Comparison of DNA encoding canine PD-L1 with known PD-L1 sequences identified the DNA sequence corresponding to the ECD domain of canine PD-L1 (SEQ ID NO: 9; which was codon optimized for CHO cells). The predicted amino acid sequence of the ECD of canine PD-L1 is SEQ ID NO: 10. DNA encoding PD-L1 ECD plus GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2695.

A DNA sequence encoding the amino acid sequence of canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 Fc (SEQ ID NO: 11) was chemically synthesized and cloned into a plasmid designated LPD2697. Canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 comprises the amino acid sequence of SEQ ID NO: 12. Table 1 contains a description of the expression plasmids mentioned above.

Table 1PLASMIDS COMPRISING DNA ENCODING PD-1 or PD-L1

<u>PLASMID NAME</u>	<u>EXPRESSED GENE</u>
P96793	Canine PD-1
LPD2726	Canine PD-1 ECD-8HIS
LPD2727	Canine PD-1 ECD-/Human IgG1 Fc
LPD2695	Canine PD-L1 ECD-8HIS
LPD2697	Canine PD-L1 ECD-/Human IgG1 Fc

Expression of PD-1 and PD-L1 proteins:

Expression plasmids encoding the PD-1ECD-HIS, PD-1ECD-Fc, PDL-1 ECD-HIS, and PD-L1ECD-Fc proteins were transfected into HEK 293 cells and the proteins were purified

from the supernatant of transfected cells using Protein A for Fc fusion proteins or Nickel (Ni^{2+}) column chromatography for HIS-tagged proteins. Purified proteins were used for: ELISA or binding assays as detailed below. Expressed proteins were analyzed by SDS-PAGE gels.

EXAMPLE 2

IDENTIFICATION OF MURINE ANTI-HUMAN MONOCLONAL ANTIBODIES THAT BIND CANINE PD-1

Confirmation of monoclonal antibodies reactivity against canine PD-1

One of the mouse monoclonal antibodies that previously had been raised against human PD-1 [hPD-1.08A, identified in US 8,354,509 B2, hereby incorporated by reference in its entirety] also was found to strongly react with canine PD-1. Purified hPD-1.08A was tested for reactivity with the HIS-tagged ECD domain of canine PD-1 by ELISA as follows: HIS-tagged canine PD-1 ECD protein is diluted to 10 $\mu\text{g/mL}$ in coating buffer (Carbonate/Bicarbonate pH 9.0) and dispensed at 100 μl /well in 96-well flat bottomed ELISA plates (NUNC). The plates are incubated at 4°C overnight. The plates are then washed three times with phosphate buffered saline containing 0.05% Tween-20 (PBST). Next, 200 μl of blocking buffer (5% skim milk in PBST) is added to each well and the plates are incubated at 37°C for 60 minutes.

The plates are then washed three times with PBST. Next, 100 μl of test monoclonal antibodies (mAbs) diluted in blocking buffer is added to the first wells of the appropriate columns. Test mAbs are then diluted two-fold to the appropriate plate position. Following incubation of the plates at 37°C for 60 minutes, the plates are washed three times with PBST. Next, 100 μl per well of a 1:2,000 dilution of a horseradish peroxidase conjugated goat anti-mouse IgG (KPL) is added to the plates, which are then incubated at 37°C for 60 minutes. Then the plates are washed three times with PBST, and 100 μl /well of 3,3',5,5' tetramethyl benzidine, (TMB) substrate (from KPL) is added to the plates. The color reaction is allowed to develop for 5-20 minutes at 37°C prior to measuring absorbance at 650nm.

CHO cells expressing canine PD-1 protein

The full length canine PD-1 gene was cloned into plasmid p96793. In this plasmid the expression of the canine PD-1 protein is driven by an hCMV promoter. CHO DXB11 cells (dhfr-) were maintained in MEM-alpha (Gibco) supplemented with 10% fetal bovine serum.

Transfection of CHO cells with plasmid p96793 was carried out in 75 cm² flasks containing approximately 6×10^6 cells by liposome-mediated gene delivery using Lipofectamine (Invitrogen). After 48 hours, cells were passaged into MEM-*alpha* medium without nucleosides, supplemented with 10% FBS and 400 µg/mL hygromycin B (selective medium). Limited-dilution cloning was performed on the pool of dhfr⁺, hygromycin resistant cells. Clones were assessed for expression of canine PD-1 by immunofluorescence assay. Briefly, cell monolayers were fixed in 96 well plates with 80% acetone. Fixed and dried cell monolayers were then incubated for 1 hour with a polyclonal goat anti-human PD-1 antibody (R&D Systems). Plates were washed with PBS, then incubated for 1 hour with a fluorescein-labeled rabbit anti-goat IgG antibody (KPL). Plates were washed with PBS. Clones exhibiting fluorescence were expanded and cell stocks were established.

Reactivity of mouse mAbs against Canine PD-1 proteins expressed on CHO cells

The reactivity of mouse anti-human PD-1 mAbs with canine PD-1 on CHO cells was determined by a cell-based assay using CHO cells that express PD-1. Briefly, the CHO cells expressing canine PD-1 were cultured to 80-100% confluency in 50 µl media (DMEM/HAM's F12, 10% FBS; "CHO Media"). Next, 50 µl of media containing various concentrations of purified mAbs were added for 1 hour at 37°C. Following three washes with PBS-TWEEN, 100 µl of goat anti-mouse horse raddish peroxidase (HRP) diluted 1:1000 in culture media was added for one hour at 37°C. After three additional washes with PBS-TWEEN, bound mAbs were visualized with a peroxidase substrate (TMB). The absorbance increase due to peroxidase activity at 450 nm was measured in a microplate reader. Color development is stopped by adding 50 µL per well of 1 M phosphoric acid.

Ligand blockade by mouse and caninized anti-PD-1 mAbs

For mouse anti-human PD-1 mAbs which react with canine PD-1, a cell-based ELISA (CELISA) assay based on the CHO cell line expressing canine PD-1 was used. Ligand blockade was confirmed using this assay in conjunction with biotinylated cPD-L1/Fc protein. Briefly, seed cPD-1 CHO cells in 96-well plates at 4×10^4 cells per well and incubate cells at 37°C for 18-24 hours till they are 95-100% confluent. Aspirate cell culture media, wash the plates 3 times with PBS + 0.05% Tween20 and 1x CHO media. Make 3-fold serial dilutions of anti-cPD1 mAbs in CHO media, starting at 30 µg/mL, and add 50 µL/well of each antibody dilution to the plate. Incubate at 37°C, 5% CO₂ with shaking for 30 min. Add 50 µL/well of

cPD-L1-Fc –biotin (2 ug/ml in CHO media stock) and continue to incubate at 37°C, 5% CO₂ with shaking for 45 min. Wash the plates six times with PBS + 0.05% Tween 20. Add 100ul/well of 1:2000 Streptavidin-Horse Raddish Peroxidase (Streptavidin-HRP) in CHO media and incubate 30-60 min at 37°C/5% CO₂. Wash the plates five times with PBS + 0.05% Tween20. Add 100 µl/well of TMB color developing substrate. Stop color development by adding 50 µl/well of 1M phosphoric acid. Measure optical density (O.D.) at A450 – A620 using an ELISA plate reader.

Cloning and identification of DNA sequences corresponding to mouse Hpd-.08A mAb

The DNA sequence of mouse VH and VL chains and the DNA sequences encoding their CDRs are identified as described US 8,354,509 [see, Table IV of US 8,354,509; provided in Table 2 directly below].

Table 2

Mouse anti-Human PD-1 CDRs from
hPD-1.08A of US 8,354,509

CDR	Heavy Chain (SEQ ID NO:)		Light Chain (SEQ ID NO:)	
	N.A.	A.A.	N.A.	A.A.
CDR-1	13	14	19	20
CDR-2	15	16	21	22
CDR-3	17	18	23	24

EXAMPLE 3

CANINIZATION OF MOUSE ANTI-HUMAN PD-1 MONOCLONAL ANTIBODIES

In order to execute the process of caninization, the DNA sequence that encodes the heavy and light chains of canine IgG were determined. The DNA and protein sequence of the canine heavy and light chains are known in the art and can be obtained by searching of the NCBI gene and protein databases. There are four known IgG subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. There are two types of light chains in canine antibodies referred to as kappa and lambda. Table 3 lists both the amino and nucleic acid sequences of

modified canine heavy (IgG-A, IgG-B, IgG-D) and light (*Kappa*) antibody chains of the present invention that comprise the murine anti-human PD-1 CDRs of Table 2.

Table 3

MODIFIED CANINE HEAVY AND LIGHT CHAIN SEQUENCES[#]

Chain type	Subtype	Nucleic Acid SEQ ID NO:	Amino Acid SEQ ID NO:
H	IgG-A	25	26
H	IgG-B	27	28
H	IgG-D	29	30
L	<i>Kappa</i> (1011)	31	32
L	<i>Kappa</i> (1022)	33	34

[#] Sequences do not include the signal sequence.

Construction of Caninized anti PD-1 Antibodies

Without being bound by any specific approach, the process of producing variants of caninized anti-PD-1 mAbs with various contents of canine and mouse sequences involved the general following scheme:

- i) Determine DNA sequence of VH and VL chains of mouse mabs
- ii) Identify the H and L chain CDRs of mouse mabs
- iii) Identify a suitable H and L chain of canine IgG
- iv) Write down the DNA sequence of canine IgG H and L chains
- v) Replace the DNA sequence encoding endogenous dog H and L chain CDRs with DNA sequences encoding the respective mouse CDRs. Also, optionally replace some canine frame residues with selected residues from the corresponding mouse frame regions.
- vi) Synthesize the DNA from step (v) and clone it into a suitable expression plasmid
- vii) Transfect plasmids into HEK 293 cells
- viii) Purify expressed antibody from HEK 293 supernatant
- ix) Test purified antibody for binding to canine PD-1

The above outlined steps resulted in a set of variant antibodies with various contents of canine and mouse sequences. The present invention identifies the caninized murine anti-human PD-1 antibodies comprising SEQ ID NO: 28 and of SEQ ID NO: 32 or 34 as having particularly tight binding with canine PD-1.

Full length canine PD-1 DNA sequence: signal sequence **underlined and in bold**

Nucleotide sequence **SEQ ID NO: 1** is without the signal sequence; and

Nucleotide sequence **SEQ ID NO: 35** includes the signal sequence.

atggggagacggcgggggccctggccgctcgtctgtgggcgtgctgcagctgggctgggtggccaggatggctcctag
actccctgacaggccctggagcccgctcaccttctccccggcgagctcacggtgcaggagggagagaacgccac
gttcacctgcagcctggccgacatccccgacagcttcgtgctcaactggtaccgctgagccccgcaaccagacg
gacaagctggccgcttccaggaggaccgcatcgagccgggacaggcgcttccgctcatgaggctgcca
acgggagggacttccacatgagcatcgtcgtgcgccctcaacgacagcgcatctacctgtgcggggccatcta
cctgcccccaacacacagatcaacgagagtccccgcgagagctctccgtgacggagagaaccctggagcccc
acacagagccccagccccaccagactcagcgccagttgcaggggctggtcatcggcgtcacgagcgtgctgg
tgggtgtcctgctactgctgctgctgacctgggtcctggccgctgtcttccccaggggccaccgaggtgectgtgt
gtgcgggagcgaggacgagcctctgaaggagggccccgatgcagcgcccgctcttccacctggactacggggagctg
gacttccagtggcgagagaagacgcccggagccccggcgccctgtgccccggagcagaccgagtatgccaccatcg
tcttcccgggcaggccggcgtccccgggcccaggccctcgccagcagcctgcaggagcccagcctccgagccc
cgaggacggacccggcctgtggcccctctga

Full length canine PD-1 Amino acid sequence: signal sequence **underlined and in bold**

Amino acid sequence **SEQ ID NO: 2** is without the signal sequence; and

Amino acid sequence **SEQ ID NO: 36** includes the signal sequence.

MGSRRGPWPLVWAVLQLGWPGWLLDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWyRLSPRNQT
DKLAAAFQEDRIEPRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPNTQINESPRAELSVTERTLEPP
TQSPSPPPRLSGQLQLGLVIGVTSVLVGVLLLLLLLTWVLAAVFPRATRGACVCGSEDEPLKEGPDAAPVFTLDYGL
DFQWREKTPEPPAPCAPEQTEYATIVFPGRPASPGRRASASSLQGAQPPSPEDGPGWLWPL

Canine PD-1 extracellular domain DNA sequence: **SEQ ID NO: 3** (Codon optimized for expression in CHO cells)

ctggattcccccgacagaccctggagccctctcaccttctccccctgccagctgaccgtccaggaaggcgagaatg
ccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggtacagactgagccccaggaacca
gaccgacaagctggccgcttccaggaggacaggatcgaaccggcaggagacaggaggtttagggctcatgaggctg
cccaacggcagggacttccacatgtccatcgtggccgcccagactgaacgactccggcatctacctgtgcggcgcta
tctacctgcccccaacaccagatcaacgagagccccaggggccgaactgagcgtgacagagagaaccctggaacc
tcccaccagagacccttccccctcctcctagactgagcggacagctgcagggcctggtg

Canine PD-1 extracellular domain: **SEQ ID NO: 4:**

LDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWyRLSPRNQTDKLAFAQEDRIEPRDRRFRVMRL
PNGRDFHMSIVAARLNDSGIYLCGAIYLPNTQINESPRAELSVTERTLEPPTQSPSPPPRLSGQLQLGLV

Canine PD-1 extracellular domain – human IgG1 Fc DNA sequence: **SEQ ID NO: 5** (Codon optimized for expression in HEK-293 cells)

ctggattcccccgacagaccctggagccctctcaccttctccccctgccagctgaccgtccaggaaggcgagaatg
ccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggtacagactgagccccaggaacca
gaccgacaagctggccgcttccaggaggacaggatcgaaccggcaggagacaggaggtttagggctcatgaggctg
cccaacggcagggacttccacatgtccatcgtggccgcccagactgaacgactccggcatctacctgtgcggcgcta
tctacctgcccccaacaccagatcaacgagagccccaggggccgaactgagcgtgacagagagaaccctggaacc
tcccaccagagacccttccccctcctcctagactgagcggacagctgcagggcctgggtgggtaccgacaaaactcac
acatgccccaccgtgcccagcacctgaactcctggggggaccgtcagtccttcttcccccaaaaacccaaggaca
ccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtt
caactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtagacaacagcacgtac
cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaaca
aagccctccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct
gcccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgac
atcgccgtggagtgaggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacg

gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt
gatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Canine PD-1 extracellular domain – human IgG1 Fc fusion protein: signal sequence

underlined and in bold: SEQ ID NO: 6; SEQ ID NO: 53 includes the signal sequence.

MNELLSSVHWSIALLLYLHAKWSQALDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWRSLSPRN
QTDKLAAFQEDRIEPRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPNTQINESPRAELSVTERTLE
PPTQSPSPPPRLSGQLQGLVGTDKHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPFSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS
VMHEALHNNHYTQKSLSLSPGK

Full length canine PD-L1 DNA sequence: signal sequence **underlined and in bold**

Nucleotide sequence **SEQ ID NO: 7 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 37 includes the signal sequence.**

atgagaatgttttagtgtctttacattcatggcctactgccatttgctaaaagcatttacgatcacagtttctaagg
acctgtatgtggtagagtatggtggcaatgtgacaatggaatgcaaattcccgggtggaaaaacagttaaacttggt
tgcactaatcgtctactgggaaatggaggataaaaaaattatacaatttgtgaatggaaaggaagacctgaaagtt
cagcacagcagctacagccagagggctcagctattgaaggaccagctcttcttggggaaggctgcgcttcagatca
cagatgtgagattgcaggatgcaggggtttactgctgcttgatcggtatggcggtgctgactacaagcggattac
tttgaaagttcatgccccgtaccgcaacatcagccaaagaatttctgtggatcctgtcacctctgaacatgaacta
atgtgtcaggtgagggttaccctgaggtgaagtcatctggacaagcagtgaccaccgagtcctgagtggcaaaa
ccaccatcactaattccaataggggaagagaagcttttcaatgtgaccagcagctgaacatcaatgcaacagctaa
tgagattttctactgcacttttcaaagatcaggtcctgaggaaaacaatactgccgagttgggtcatcccagaacga
ctgcccgttccagcaagtgaagaggactcattcatgattctgggaccttccctgttgcttcttgggtgtagtctctgg
cagtcactttctgtctaaaaaacatgggagaatgatggatgtggaaaaatgttgcaccgcagataggaactcaaa
gaaacgaaatgatatacaatttgaagagacataa

Full length canine PD-L1: signal sequence **underlined and in bold**

Amino acid sequence **SEQ ID NO: 8 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 38 includes the signal sequence.**

MRMFSVFETFMAYCHLLKAFTITVSKDLVVEYGGNVMTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKV
QHSSYSQRAQLLKDQLFLGKAALQITDVRLQDAGVYCCILIGYGADYKRITLKVHAPYRNISQRI SVD PVTSEHEL
MCQAEGYPEAEVIWTSDDHRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRS GP EENNTAELVIPER
LPVPASERTHFMI LGPFLLLLGVVLAVTFCLKKHGRMMDVEKCCTRDRNSKKRNDIQFEET

Canine PD-L1 extracellular domain DNA sequence: **SEQ ID NO: 9** (Codon optimized for expression in CHO cells)

ttaccatcacccgtgtccaaggacctgtacgtggtcgagtacggcggaatgtgaccatggagtgcaagttccccg
tggaagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggacaagaagatcatccagttcgtgaa
cggcaaggaggacctgaaggtgcagcactccagctactcccagagagcccagctgctgaaggaccagctgttcctg
ggcaaggccgccctgcagatcacccgacgtgagactgcaggacgccggcgtgtattgctgctgactcggtacggag
gcgcccactacaagaggatcacccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcc
cgtgaccagcgagcagcagctgatgtgccaaagccgagggtatcccaggccgaagtgatctggaccagcagcgac
cacagggtcctgagcggcaagaccaccatcaccaacagcaacaggaggagaagctgttcaacgtgaccagcacc
tcaacatcaacgccaccgccaacgagatcttctactgcaccttccagaggagcgccccgaagagaacaacaccgc
cgagctggtgatccccgagagactgcctgtgctgctgcagcgagaggaccac

Canine PD-L1 extracellular domain protein: **SEQ ID NO: 10**

FTITVSKDLVVEYGGNVMTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQRAQLLKDQLFL
GKAALQITDVRLQDAGVYCCILIGYGADYKRITLKVHAPYRNISQRI SVD PVTSEHEL MCQAEGYPEAEVIWTSDD
HRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRS GP EENNTAELVIPERLPVPASERTH

Canine PD-L1 extracellular domain – human IgG1 Fc DNA sequence: **SEQ ID NO: 11**
(Codon optimized for expression in HEK-293 cells)

tttaccatcacccgtgtccaaggacctgtacgtggctcgagtacggcggcaatgtgaccatggagtgcaagttccccg
 tggagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggacaagaagatcatccagttcgtgaa
 cggcaaggaggacctgaaggtgcagcactccagctactcccagagagcccagctgctgaaggaccagctgttcctg
 ggcaaggccgccttcgagatcacccagctgagactgcaggacgccggcgtgtattgctgcctgatcggctacggag
 gcgccgactacaagaggatcacccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcc
 cgtgaccagcgcagcagctgatgtgccaagccgagggctatcccagggccgaagtgatctggaccagcagcgac
 cacagggctcctgagcggcaagaccaccatcaccaacagcaacagggaggagaagctgttcaacgtgaccagcacc
 tcaacatcaacgccaccgcccaacgagatcttctactgcaccttccagaggagcggccccgaagagaacaacaccgc
 cgagctgggtgatccccgagagactgcctgtgcttgccagcgagaggacccacggtaccgacaaaactcacacatgc
 ccacgtgcccagcacctgaactcctggggggaccgtcagcttctcttccccccaaaacccaaggacaccctca
 tgatctcccgaccctgaggtcacatgcgtgggtgggacgtgagccacgaagaccctgaggtcaagttcaactg
 gtacgtggacggcgtggaggtgcataatgccaaagcaaaagccgcgggaggagcagtacaacagcacgtaccgtgtg
 gtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
 tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccc
 atccccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgcc
 gtggagtgaggagacaaatggggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
 tcttcctctacagcaagctcacctgggacaagagcaggtggcagcaggggaacgtcttctcatgctcctgatgca
 tgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Canine PD-L1 extracellular domain – human IgG1 Fc fusion protein: SEQ ID NO: 12

FTITVSKDLVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQRAQLLKDQLFL
 GKAAALQITDVRQLDAGVYCCCLIGYGADYKRITLKVHAPYRNISQRI SVDPVTSEHELMCQAEGYPEAEVIWTS
 HRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQSRGPEENNTAELVIPERLPVPASERTHGTDKHTC
 PPCPAPELLGGPSVFLFPPPKPDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
 VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

08A VH : CDR H1 DNA: SEQ ID NO: 13:

agttattatc tgtac

08A VH : CDR H1 protein: SEQ ID NO: 14:

Ser Tyr Tyr Leu Tyr

08A VH : CDR H2 DNA: SEQ ID NO: 15:

gggggttaatc ctagtaatgg tggactaac ttcagtgaga agttcaag

08A VH : CDR H2 protein: SEQ ID NO: 16:

Gly Val Asn Pro Ser Asn Gly Gly Thr Asn Phe Ser Glu Lys Phe
 Lys

08A VH : CDR H3 DNA: SEQ ID NO: 17:

agggattcta actacgacgg gggctttgac tac

08A VH : CDR H3 protein: SEQ ID NO: 18:

Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr

08A VL : CDR L1 DNA: SEQ ID NO: 19:

agggccagca aaagtgtcag tacatctggc tttagttatt tgcac

08A VL : CDR L1 protein: SEQ ID NO: 20:

Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His

08A VL : CDR L2 DNA: SEQ ID NO: 21:

cttgcatcca acctagagtc t

08A VL : CDR L2 protein: SEQ ID NO: 22:

Leu Ala Ser Asn Leu Glu Ser

08A VL : CDR L3 DNA: SEQ ID NO: 23:

cagcacagttt gggagcttcc gctcacg

08A VL : CDR L3 protein: SEQ ID NO: 24:

Gln His Ser Trp Glu Leu Pro Leu Thr

Caninized Murine Anti-Human PD-1 Antibody 08A

canVH-canIgGB-Fc (12G8 signal sequence underlined and in bold): HEAVY CHAIN

Nucleotide sequence SEQ ID NO: 27 is without the signal sequence; and

Nucleotide sequence SEQ ID NO: 41 includes the signal sequence.

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
CCGGCGGCGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTACCAGCTA
CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCCTGAGCGTGGACAAGGCCAAGAACCAGCCTACATGCAGC
TGAAGTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCGA
CTACTGGGGACAGGGAACCCCTGCTGACCGTGTCCAGCGCTTCCACAACCGCGCCATCAGTCTTTCCGTTGGCCCCA
TCATGCGGGTCGACGAGCGGATCGACTGTGGCCCTGGCGTGCTTGGTGTGCGGGATACTTTCCCGAACCCGTCACGG
TCAGCTGGAAGTCCGGATCGCTTACGAGCGGTGTGCATACGTTCCCTCGGTCTTGCAATCATCAGGGCTCTACTC
GCTGTGCGAGCATGGTAACGGTGCCCTCATCGAGGTGGCCCTCCGAAACGTTACATGTAACGTAGCACATCCAGCC
TCCAAAACCAAGGTGGATAAACCCGTGCCGAAAAGAGAGAATGGGCGGGTGCCTCGACCCCTGATTGCCCAAGT
GTCCGGCTCCGGAATGCTCGGTGGACCCCTCAGTGTTTATCTTCCCTCCGAAGCCCAAGGACACTCTGCTGATCGC
GCGCACTCCAGAAGTAACATGTGTAGTGGTGGACCTTGATCCCGAGGACCCCGAAGTCCAGATCTCCTGGTTTGTA
GATGGGAAACAGATGCAGACCGCAAAACTCAACCCAGAGAGGAGCAGTTCAACGGAACATACCGAGTGGTATCCG
TCCTTCCGATTGGCCACCAGGACTGGTTGAAAGGGAAGCAGTTTACGTGTAAAGTCAACAATAAGGCGTTGCCTAG
CCCTATTGAGCGGACGATTTCCGAAAGCTAGGGGACAGGCCACCAGCCATCGGTCTATGTCTTCCGCCTTCCCGC
GAGGAGCTCTCGAAGAATACAGTGAGCCTTACATGCCTCATTAAGGATTTCTTCCCGCCTGATATCGACGTAGAGT
GGCAATCAAAACGGTCAACAGGAGCCGGAATCCAAGTATAGAACCCTCCGCCCCAGCTTGACGAGGACGGATCATA
CTTTTGTATTCAAACTGTGCGGTGGATAAGAGCCGGTGGCAGAGAGGTGACACCTTCATCTGTGCGGTGATGCAC
GAAGCACTCCATAATCACTACACCCAAGAGAGCCTCTCGCATTTCCCCCGGAAAGTGA

Amino acid sequence SEQ ID NO: 28 is without the signal sequence; and

Amino acid sequence SEQ ID NO: 42 includes the signal sequence.

MAVLGLLFLCLVTFPSCVLSEVQLVQSGGDLVKPGGSRVLSVASGYTFTSYLYWVRQAPGKGLQWIGGVNPSNGG
TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLTIVSSASTTAPSVFPLAP
SCGSTSGSTVALACLVSIFYPEPVTVSWNSGLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPA
SKTKVDKPVKPRENRRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDILLIARTPEVTCVVVDLDPEDPEVQISWV
DGKQMOTAKTQPREEQFNQTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPR
EELSKNTVSLTCLIKDFFPPDIDVEWQSNQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMH
EALHNHYTQESLSHSPGK

canVH-canIgGA-Fc (12G8 signal sequence underlined and in bold): HEAVY CHAIN

Nucleotide sequence SEQ ID NO: 25 is without the signal sequence; and

Nucleotide sequence SEQ ID NO: 39 includes the signal sequence.

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
CCGGCGGCGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTACCAGCTA
CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCCTGAGCGTGGACAAGGCCAAGAACCAGCCTACATGCAGC
TGAAGTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCGA
CTACTGGGGACAGGGAACCCCTGCTGACCGTGTCCAGCGCTTCCACAACGGCTCCGTCGGTGTTCCTTCCCTGGCACCT
AGCTGCGGGTCGACCTCGGGTAGCACAGTGGCGCTGGCGTGTTTGGTGTGCGGGATACTTTCCCGAGCCGGTAACGG
TGTCATGGAAGTCAAGGTCACTTACATCAGGAGTCCATACTTTTCCGTCCGTGCTGCAGTCAAGCGGCTTGCAATTC
ACTGTCTCGATGGTGACGGTGCCCTTCGTCGAGGTGGCCAGCGAAACGTTCACTTGTAACGTAGTACACCCGGCC

TCCAACACGAAAGTCGATAAACCGGTATTCAATGAGTGCAGATGTACAGACACCCCTCCCTGTCCGGTACCCGAAC
 CCCTTGGAGGGCCGAGCGTCCCTCATCTTCCCTCCCAAGCCAAAAGACATCTTGCGCATTACGAGGACACCAGAAGT
 CACGTGCGTAGTGCTTGATCTCGGTAGAGAAGATCCCGAGGTCCAGATCTCGTGGTTTGTGGATGGAAAGGAGGTC
 CACACCGCAAAGACTCAGTCGCGCGAGCAGCAGTTCAATGGCACGTATCGGGTCGTGAGCGTGCTTCCTATCGAGC
 ATCAGGACTGGCTCACCGGGAAGGAGTTCAAATGCCGGGTCAATCATATCGACCTCCCGTCACCAATCGAGCGGAC
 CATCTCGAAGGCTAGAGGAAGGGCGCACAAACCTTCGGTCTATGTGCTTCCCCCATCGCCCAAAGAGCTTTCTCTCG
 TCGGATACGGTGTCCATTACATGCTTGATTAAGGACTTCTATCCTCCTGATATTGATGTGGAATGGCAATCAAACG
 GACAGCAGGAGCCGGAACGCAAGCACCGAATGACCCACCGCAATTGGACGAAGATGGTAGCTACTTTCTCTACTC
 AAAGCTCTCAGTCGACAAATCCCGATGGCAGCAGGGAGATCCCTTCACTTGCGCCGTGATGCACGAGACACTCCAA
 AATCATTACACGGACCTTTCGTTGAGCCACTCGCCCGGAAAG

Amino acid sequence **SEQ ID NO: 26** is without the signal sequence; and
 Amino acid sequence **SEQ ID NO: 40** includes the signal sequence.

MAVLGLLFLCLVTFPSCVLSEVQLVQSGGDLVKPGGSVRLSCVASGYTFTSYLYWVRQAPGKGLQWIGGVNPSNNG
 TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLLTVSSASTTAPSVFPLAP
 SCGSTSGSTVALACLVSIFYPEPVTVSWNSGSLTSGVHTFPSVLQSSGLHSLSSMTVPSSRWPSETFTCNVHHPA
 SNTKVDKPVFNECRCTDTPPCPVPEPLGGPSVLIFFPKPKDILRITRTPVETCVVLDLGGREDPEVQISWFDGKEV
 HTAKTQSREQQFNGTYRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPRIERTISKARGRAHKPSVYVLPSPKELSS
 SDTVSITCLIKDFYPPDIDVEWQSNQQEPERKHRMTPQLDEDEGSYFLYSKLSVDKSRWQQGDPFTCAVMHETLQ
 NHYTDLSLSHSPGK

canVH-canIgGD-Fc (12G8 signal sequence **underlined and in bold**): HEAVY CHAIN
 Nucleotide sequence **SEQ ID NO: 29** is without the signal sequence; and
 Nucleotide sequence **SEQ ID NO: 43** includes the signal sequence.

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
 CCGGCGCGGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTACCAGCTA
 CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
 ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCTGAGCGTGGACAAGGCCAAGAACACCGCCTACATGCAGC
 TGAATCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCTGA
 CTACTGGGGACAGGGAACCTGTGACCGTGTCCAGCGCTTCAACCACAGCGCCGAGCGTGTTCCTCTGGCGCCG
 TCGTGCGGTTCACCTCGGGATCAACAGTGGCCCTCGCCTGTCTCGTGAGCGGATACTTTCCGGAGCCTGTACGG
 TGTCGTGGAATAGCGGATCACTCACGTGGGCGTGCATACTTTTCCATCCGTCTTGCAATCGAGCGGATTGTACTC
 ACTCTCCTCAACCGTCACTGTCCCCCTCGTCGCGCTGGCCCTCGGAGACTTTTACGTGCAATGTAGTCCATCCGGCG
 AGCAACACGAAGGTGACAAGCCCGTACCCAAGGAATCAACATGCAAGTGCATCTCGCCCTGTCCCGTCCCCGAAT
 CCCTTGGTGGCCCCCTCAGTGTTTATCTTCCCTCCGAAGCCTAAAGACATCTTGAGAATCACAGAACACCGGAAAT
 CACGTGTGTGGTCTTACTTGGGACGCGAGGACCTTGAGGTACAAATCTCGTGGTTTGTGGACGGGAAAGAGGTG
 CACACGCAAAAGACACAACACCGCAGCAGTAACTCAACGTACAGGAGTAGTATCCGTAATCTCCATTGAAC
 ACCAGGATTGGCTCACCGGTAAAGAATTCAAATGCCGAGTGAATCACATCGGGCTTCTAGCCCAATTGAGCGGAC
 GATTTCCAAAGCTAGGGGTACGGCCACCAGCCGAGCGTATACGTGTTGCCGCCCTCCCCGAAGGAGCTGTATCG
 TCAGATACGGTAACGTTGACGTGTCTGATCAAAGATTTCTTTCTCCCGAAATTGATGTGGAATGGCAAAGCAATG
 GGCAGCCCGAGCCGAGTCAAAGTACCATACTACTGCACCACAGCTGGACGAAGATGGATCGTATTTCTCTACTC
 GAAACTGTCCGTGGATAAGTCCCGGTGGCAGCAAGGGGACACCTTCACTTGCGCGGTATGCACGAGGCACCTTCA
 AACCATAACGGACTTGAGCCTCTCGCATTCGCCAGGGAAG

Amino acid sequence **SEQ ID NO: 30** is without the signal sequence; and
 Amino acid sequence **SEQ ID NO: 44** includes the signal sequence.

MAVLGLLFLCLVTFPSCVLSEVQLVQSGGDLVKPGGSVRLSCVASGYTFTSYLYWVRQAPGKGLQWIGGVNPSNNG
 TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLLTVSSASTTAPSVFPLAP
 SCGSTSGSTVALACLVSIFYPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMTVPSSRWPSETFTCNVHHPA
 SNTKVDKPVFKESTCKCISPCPVPESLGGPSVFIFFPKPKDILRITRTPETITCVVLDLGGREDPEVQISWFDGKEV
 HTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPSPKELSS
 SDTVTLTCLIKDFPPEIDVEWQSNQQPEPESKYHTTAPQLDEDEGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQ
 NHYTDLSLSHSPGK

canVL-canKappa (1022)xHGF signal sequence **underlined and in bold**: LIGHT CHAIN
 Nucleotide sequence SEQ ID NO: 33 is without the signal sequence; and
 Nucleotide sequence SEQ ID NO: 47 includes the signal sequence.

ATGGATATGAGAGTACCTGCACAACCTTCTGGGATTGCTGCTTCTTTGGCTGAGAGGGGGCCCGCTGCGATATCGTCC
 TGACCCAGACCCCTCCTAGCCTGTCCGTGAGCCCTGGAGAACCCGCCAGCATCAGCTGCAGGGCCTCCAAGAGCGT
 GAGCACCAGCGGCTTCAGCTACCTGCACTGGTACAGGCAGAAGCCCGGACAGCCTCCTCAGCTGCTGATCTTCCTG
 GCCAGCAACCTGGAGAGCGGCGTGCCGTGACAGGTTTAGCGGAAGCGGCAGCGGCACCGACTTCACACTGAGGATCT
 CCAGGGTGGAAGCCGACGACGCCGGAGTGTACTACTGCCAGCACAGCTGGGAAGTGGCCCTGACCTTCGGCCAGGG
 CACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCAT
 ACGGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACG
 GGGTAATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTC
 AACCTTGACGATGTCAAGCACCAGATATCTTAGCCATGAGCTGTATTCTGTGCGAGATCACCCACAAGTCCCTCCCC
 TCCACTCTTATCAAATCCTTTCAGCGGTGCGAATGTCAGCGGGTCGAT

Amino acid sequence SEQ ID NO: 34 is without the signal sequence; and
 Amino acid sequence SEQ ID NO: 48 includes the signal sequence.

MDMRVPAQLLGLLLLWLRGARCDIVLTQTTPPSLSVSPGEPASISCRASKSVSTSGFSYLHWYRQKPGQPPQLLIFL
 ASNLESGVPDRFSGSGSGTDFTLRISRVEADDAGVYYCQHSWELPLTFGQGTKVEIKRNDAPAVYLFQPSPDQLH
 TGSASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYLSSTLTMSSTEYLSHELYSCEITHKSLP
 STLIKSFRSECQRVD

canVL-canKappa (1011)(xHGF signal sequence **underlined and in bold**:LIGHT CHAIN
 Nucleotide sequence SEQ ID NO: 31 is without the signal sequence; and
 Nucleotide sequence SEQ ID NO: 45 includes the signal sequence.

ATGGATATGAGAGTACCTGCACAACCTTCTGGGATTGCTGCTTCTTTGGCTGAGAGGGGGCCCGCTGCGATATCGTCC
 TGACCCAGACCCCTCTGAGCCTGTCCGTGAGCCCTGGAGAACCCGCCAGCATCAGCTGCAGGGCCTCCAAGAGCGT
 GAGCACCAGCGGCTTCAGCTACCTGCACTGGTACAGGCAGAAGCCCGGACAGAGCCCTCAGCTGCTGATCTTCCTG
 GCCAGCAACCTGGAGAGCGGCGTGCCGTGACAGGTTTAGCGGAAGCGGCAGCGGCACCGACTTCACACTGAGGATCT
 CCAGGGTGGAAGCCGACGACGCCGGAGTGTACTACTGCCAGCACAGCTGGGAAGTGGCCCTGACCTTCGGCCAGGG
 CACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCAT
 ACGGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACG
 GGGTAATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTC
 AACCTTGACGATGTCAAGCACCAGATATCTTAGCCATGAGCTGTATTCTGTGCGAGATCACCCACAAGTCCCTCCCC
 TCCACTCTTATCAAATCCTTTCAGCGGTGCGAATGTCAGCGGGTCGAT

Amino acid sequence SEQ ID NO: 32 is without the signal sequence; and
 Amino acid sequence SEQ ID NO: 46 includes the signal sequence.

MDMRVPAQLLGLLLLWLRGARCDIVLTQTPLSLSVSPGEPASISCRASKSVSTSGFSYLHWYRQKPGQSPQLLIFL
 ASNLESGVPDRFSGSGSGTDFTLRISRVEADDAGVYYCQHSWELPLTFGQGTKVEIKRNDAPAVYLFQPSPDQLH
 TGSASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYLSSTLTMSSTEYLSHELYSCEITHKSLP
 STLIKSFRSECQRVD

EXAMPLE 4

MUTANT CANINE IgG-B ANTIBODIES SPECIFIC TO PD-1

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and

kappa. However, besides binding and activating of canine immune cells, a canine or caninized antibody against PD-1 optimally has two attributes:

1. lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
2. be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has a high level of ADCC activity. IgG-C also has considerable ADCC activity. On the other hand, IgG-A binds weakly to protein A, but displays undesirable ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D display no ADCC activity. The present invention overcomes this difficulty by providing mutant canine IgG-B antibodies specific to PD-1; such antibodies lack effector functions such as ADCC and can be easily purified using industry standard protein A chromatography. The exact modifications are shown in Figure 4.

The IgG-B variants with reduced effector functions described encompass a first IgG-B variant in which a lysine (D 277) and an asparagine (N 325) residue is each mutated to an alanine residue [cIgGB(-) ADCC], a second variant in which the hinge region of IgG-B is replaced by the hinge region of IgG-D [cIgGB(+) D-hinge], and a third variant in which the hinge region of IgG-B is replaced with the hinge region of IgG-A [cIgGB(+) A-hinge]. Additionally, the second and third variants also include replacement of the same lysine and asparagine residues of the first variant with an alanine residue. The numbering of the lysine and asparagine residues mutated in this invention is based on the numbering scheme described for canine IgG heavy chains in Tang *et al.*, [*Vet Immunol and Immunopathol*, 80:259-270 (2001)].

Canine IgGB wt

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APPEMLGGPSVFIFFPKPKDTLLIARTPEVTCVVVD
LDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARG
QAHQPSVYVLPSPREELSKNTVSLTCLIKDFFPPDIDVEWQSNQQEPEPKYRTTPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO:49

Canine IgGB(+)A-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**FNECRCTDTPPCP**APPEMLGGPSVFIFFPKPK**A**TLLIARTPEVTCVVVDLDPED
PEVQISWFVDGKQMQTAKTQPREEQ**F**AGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQP

SVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRG
DTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO: 50

Canine IgGB(+) D-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKESTCKCISPCP**APPEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVVDLDPED
PEVQISWFDGKQMQTAKTQPREEQF**AG**TYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQP
SVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRG
DTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO: 51

Canine IgGB(-) ADCC

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APPEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVVD
LDPEDPEVQISWFDGKQMQTAKTQPREEQF**AG**TYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARG
QAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNQQEPESKYRTTPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO: 52

SEQUENCE LISTING TABLE 1

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
1	√		Canine PD-1 Full Length	18		√	hPD-1 CDR3 of V _H
2		√	Canine PD-1 Full Length	19	√		hPD-1 CDR1 of V _L
3	√		Canine PD-1 ECD	20		√	hPD-1 CDR1 of V _L
4		√	Canine PD-1 ECD	21	√		hPD-1 CDR2 of V _L
5	√		Canine PD-1 - Human IgG1	22		√	hPD-1 CDR2 of V _L
6		√	Canine PD-1 - Human IgG1	23	√		hPD-1 CDR3 of V _L
7	√		Canine PD-L1 Full Length	24		√	hPD-1 CDR3 of V _L
8		√	Canine PD-L1 Full Length	25	√		IgG-A Heavy
9	√		Canine PD-L1 ECD	26		√	IgG-A Heavy
10		√	Canine PD-L1 ECD	27	√		IgG-B Heavy
11	√		Canine PD-L1 - Human IgG1	28		√	IgG-B Heavy
12		√	Canine PD-L1 - Human IgG1	29	√		IgG-D Heavy
13	√		hPD-1 CDR1 of V _H	30		√	IgG-D Heavy
14		√	hPD-1 CDR1 of V _H	31	√		<i>Kappa</i> (Light) (1011)

15	√		hPD-1 CDR2 of V _H	32		√	<i>Kappa</i> (Light) (1011)
16		√	hPD-1 CDR2 of V _H	33	√		<i>Kappa</i> (Light) (1022)
17	√		hPD-1 CDR3 of V _H	34		√	<i>Kappa</i> (Light) (1022)

SEQUENCE LISTING TABLE 2 (with the LEADER SEQUENCES)

<u>ID</u>	<u>N.A.</u>	<u>A.A.</u>	<u>Description</u>	<u>ID</u>	<u>N.A.</u>	<u>A.A.</u>	<u>Description</u>
35	√		Canine PD-1 Full Length	42		√	IgG-B Heavy
36		√	Canine PD-1 Full Length	43	√		IgG-D Heavy
37	√		Canine PD-L1 Full Length	44		√	IgG-D Heavy
38		√	Canine PD-L1 Full Length	45	√		<i>Kappa</i> (Light) (1011)
39	√		IgG-A Heavy	46		√	<i>Kappa</i> (Light) (1011)
40		√	IgG-A Heavy	47	√		<i>Kappa</i> (Light) (1022)
41	√		IgG-B Heavy	48		√	<i>Kappa</i> (Light) (1022)
53		√	Canine PD-1 - Human IgG1 Full Length				

SEQUENCE LISTING TABLE 3

<u>ID</u>	<u>N.A.</u>	<u>A.A.</u>	<u>Description</u>	<u>ID</u>	<u>N.A.</u>	<u>A.A.</u>	<u>Description</u>
49	√		cIgGB wt	51	√		cIgGB(+)D-hinge
50	√		cIgGB(+)A-hinge	52	√		cIgGB(-)ADCC

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (*e.g.* Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (*e.g.* Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

CLAIMS

1. An isolated caninized antibody or an antigen binding fragment thereof that specifically binds Programmed Cell Death Receptor 1 (PD-1), wherein said antibody comprises a canine IgG heavy chain and a canine *kappa* light chain; wherein the canine *kappa* light chain comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and the canine IgG heavy chain comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3);

wherein the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 22, the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18;

wherein the antibody and the antigen binding fragment thereof bind canine PD-1 and block the binding of canine PD-1 to canine Programmed Cell Death Ligand 1 (PD-L1).

2. The isolated caninized antibody or an antigen binding fragment thereof of claim 1, wherein the IgG heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 28 and SEQ ID NO: 30.

3. The isolated caninized antibody or an antigen binding fragment thereof of claims 1 or 2, wherein the *kappa* light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 32 and SEQ ID NO: 34.

4. The isolated caninized antibody or an antigen binding fragment thereof of claim 1, 2, or 3, comprising the amino acid sequence of SEQ ID NO: 28 and of SEQ ID NO: 34.

5. An isolated nucleic acid that encodes the light chain of the caninized antibody of claims 3 or 4.

6. An isolated nucleic acid that encodes the heavy chain of the caninized antibody of claims 2 or 4.

7. An isolated nucleic acid of claims 5 or 6 that comprises one or more nucleotide sequences selected from the group consisting of SEQ ID NOs: 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33.
8. The isolated nucleic acid of claim 7 that comprises the nucleotide sequence of SEQ ID NO: 27 or SEQ ID NO: 33.
9. An expression vector comprising the isolated nucleic acid of claims 5, 6, 7, or 8.
10. A host cell comprising one or more expression vectors of claim 9.
11. A pharmaceutical composition comprising the antibody or antigen binding fragment of claims 1, 2, 3, or 4 and a pharmaceutically acceptable carrier or diluent.
12. A method of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of Claim 11.
13. The method of claim 12, wherein said method is used for:
 - a. the treatment of cancer;
 - b. the treatment of an infection or infectious disease; or
 - c. as a vaccine adjuvant.
14. A method of producing a caninized antibody or antigen binding fragment thereof that specifically binds PD-1 comprising:
 - a. culturing the host cell of claim 10 in culture medium under conditions wherein the nucleic acid is expressed, thereby producing a polypeptide comprising the light chain variable regions and a polypeptide comprising the heavy chain variable regions; and
 - b. recovering the polypeptides from the host cell or culture medium.

15. Use of an effective amount of the antibody or antigen binding fragment of claims 1, 2, 3 or 4 in increasing the activity of an immune cell in a subject in need thereof.
16. The use of claim 15 wherein said use is for:
- d. the treatment of cancer;
 - e. the treatment of an infection or infectious disease; or
 - f. as a vaccine adjuvant.
17. Use of the antibody or antigen binding fragment of claims 1, 2, 3 or 4 in the manufacture of a medicament for increasing the activity of an immune cell in a subject.
18. The use of claim 17, wherein the medicament is for:
- a. the treatment of cancer;
 - b. the treatment of an infection or infectious disease; or
- as a vaccine adjuvant.
19. A caninized antibody or antigen binding fragment thereof produced by performing the method of claim 14.

Intervet International B.V.

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

Figure 1. Reactivity of mouse 08A mAb against His-tagged extracellular domain of canine PD-1.

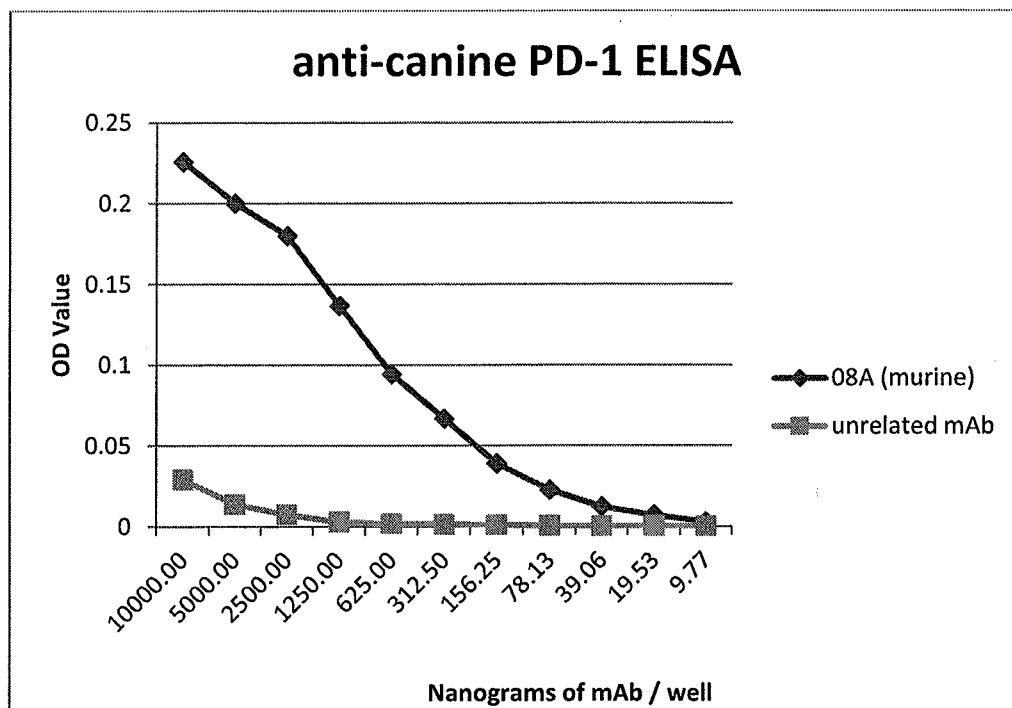


Figure 2: Reactivity of mouse 08A mAbs against Canine PD-1 proteins expressed on CHO cells using CELISA. Murine 08A antibody and its caninized variants react with PD-1 in a dose dependent manner.

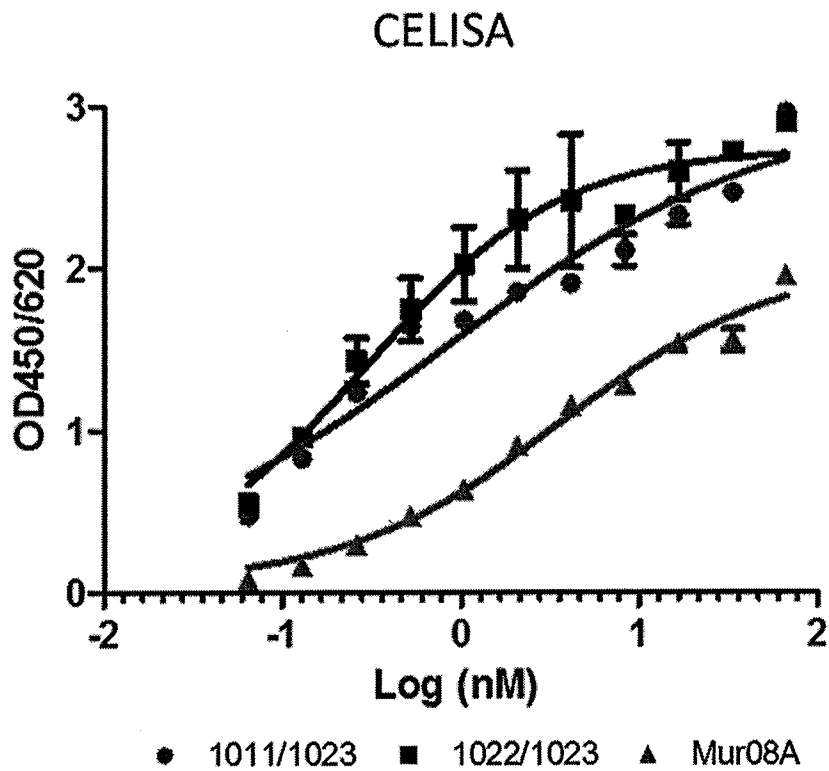


Figure 3. ligand blockade by mouse and caninized antibodies. Murine 08A and its caninized variants block binding of canine PD-1 to PD-1 expressed on CHO cell surface.

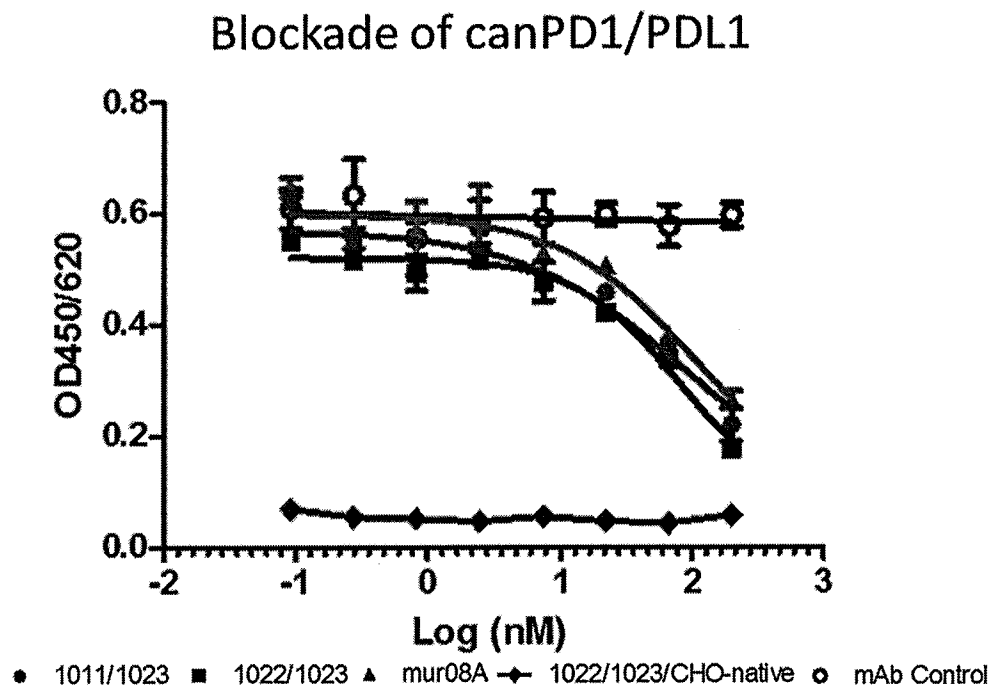


Figure 4. Alignment of canine IgGB CHs lacking ADCC function

cIgGB wt	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (+) A-hinge	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (+) D-hinge	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (-) ADCC	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
cIgGB	MLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (+) A-hinge	MLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (+) D-hinge	MLGGPSVFI	PPKPKATLLIARTPEV
cIgGB (-) ADCC	MLGGPSVFI	PPKPKATLLIARTPEV
canIgGB	SREHLSKNTVSLTCLIKDEPPEDIVAEWQSGQPEPSKYRTTQESLSHSPGK	
cIgGB (+) A-hinge	SREHLSKNTVSLTCLIKDEPPEDIVAEWQSGQPEPSKYRTTQESLSHSPGK	
cIgGB (+) D-hinge	SREHLSKNTVSLTCLIKDEPPEDIVAEWQSGQPEPSKYRTTQESLSHSPGK	
cIgGB (-) ADCC	SREHLSKNTVSLTCLIKDEPPEDIVAEWQSGQPEPSKYRTTQESLSHSPGK	
cIgGB wt	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
	TCVVDLPDPEVQISWFDGKQMTAKTQPREEQFAGTYRVVSVLP	PIGHQDWLKGKQFTCKVNNKALPSP
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cIgGB (+) A-hinge	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
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	KLSDKSRWQRGDTFTICAVMHEALHNHYTQESLSHSPGK	
cIgGB (+) D-hinge	SASTTAPSVFPLAPSCGSGTVALACLVSGYFFPEPVTVSWNSGSLTSGVHTFPVLSQSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVKRENGVVRPPDCPKCPAPEMLGGPSVFI	PPKPKATLLIARTPEV
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Bartels-Morozov, Denise
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Asp Phe His Met Ser Ile Val Ala Ala Arg Leu Asn Asp Ser Gly Ile
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Ser Pro Arg Ala Glu Leu Ser Val Thr Glu Arg Thr Leu Glu Pro Pro
115 120 125

Thr Gln Ser Pro Ser Pro Pro Arg Leu Ser Gly Gln Leu Gln Gly
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Ala Cys Val Cys Gly Ser Glu Asp Glu Pro Leu Lys Glu Gly Pro Asp
180 185 190

Ala Ala Pro Val Phe Thr Leu Asp Tyr Gly Glu Leu Asp Phe Gln Trp
195 200 205

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 50 55 60

Pro Gly Arg Asp Arg Arg Phe Arg Val Met Arg Leu Pro Asn Gly Arg
 65 70 75 80

Asp Phe His Met Ser Ile Val Ala Ala Arg Leu Asn Asp Ser Gly Ile
 85 90 95

Tyr Leu Cys Gly Ala Ile Tyr Leu Pro Pro Asn Thr Gln Ile Asn Glu
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atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag 600
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg 660
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac 720
tggtgaatg gcaaggagta caagtgaag gtctccaaca aagccctccc agccccatc 780
gagaaaacca tctccaaagc caaaggcgag ccccgagaac cacaggtgta caccctgccc 840
ccatccccgg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggcttc 900
tatcccagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag 960
accacgcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcaa gctcaccgtg 1020
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg 1080
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtaaata 1128

<210> 6
<211> 375
<212> PRT
<213> Artificial Sequence

<220>
<223> canine and human

<400> 6

Leu Asp Ser Pro Asp Arg Pro Trp Ser Pro Leu Thr Phe Ser Pro Ala
1 5 10 15

eolf-seql.txt

Gln Leu Thr Val₂₀ Gln Glu Gly Glu Asn₂₅ Ala Thr Phe Thr Cys₃₀ Ser Leu
Ala Asp Ile₃₅ Pro Asp Ser Phe Val₄₀ Leu Asn Trp Tyr Arg₄₅ Leu Ser Pro
Arg Asn₅₀ Gln Thr Asp Lys Leu₅₅ Ala Ala Phe Gln Glu₆₀ Asp Arg Ile Glu
Pro Gly Arg Asp Arg Arg₇₀ Phe Arg Val Met Arg₇₅ Leu Pro Asn Gly Arg₈₀
Asp Phe His Met Ser₈₅ Ile Val Ala Ala Arg₉₀ Leu Asn Asp Ser Gly₉₅ Ile
Tyr Leu Cys Gly₁₀₀ Ala Ile Tyr Leu Pro₁₀₅ Pro Asn Thr Gln Ile Asn Glu
Ser Pro Arg₁₁₅ Ala Glu Leu Ser Val₁₂₀ Thr Glu Arg Thr Leu₁₂₅ Glu Pro Pro
Thr Gln₁₃₀ Ser Pro Ser Pro₁₃₅ Pro Arg Leu Ser Gly₁₄₀ Gln Leu Gln Gly
Leu Val Gly Thr Asp Lys₁₅₀ Thr His Thr Cys Pro₁₅₅ Pro Cys Pro Ala Pro₁₆₀
Glu Leu Leu Gly Gly₁₆₅ Pro Ser Val Phe Leu₁₇₀ Phe Pro Pro Lys Pro₁₇₅ Lys
Asp Thr Leu Met₁₈₀ Ile Ser Arg Thr Pro₁₈₅ Glu Val Thr Cys Val₁₉₀ Val Val
Asp Val Ser₁₉₅ His Glu Asp Pro Glu₂₀₀ Val Lys Phe Asn Trp₂₀₅ Tyr Val Asp
Gly Val₂₁₀ Glu Val His Asn Ala₂₁₅ Lys Thr Lys Pro Arg₂₂₀ Glu Glu Gln Tyr
Asn Ser Thr Tyr Arg Val₂₃₀ Val Ser Val Leu Thr₂₃₅ Val Leu His Gln Asp₂₄₀
Trp Leu Asn Gly Lys₂₄₅ Glu Tyr Lys Cys Lys₂₅₀ Val Ser Asn Lys Ala₂₅₅ Leu
Pro Ala Pro Ile₂₆₀ Glu Lys Thr Ile Ser₂₆₅ Lys Ala Lys Gly Gln₂₇₀ Pro Arg
Glu Pro Gln₂₇₅ Val Tyr Thr Leu Pro₂₈₀ Pro Ser Arg Asp Glu₂₈₅ Leu Thr Lys

eolf-seql.txt

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
290 295 300

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
305 310 315 320

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
325 330 335

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
340 345 350

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
355 360 365

Leu Ser Leu Ser Pro Gly Lys
370 375

<210> 7
<211> 816
<212> DNA
<213> Canis familiaris

<400> 7
tttacgatca cagtttctaa ggacctgtat gtggtagagt atggtggcaa tgtgacaatg 60
gaatgcaaat tcccgggtga aaaacagtta aacttgtttg cactaatcgt ctactgggaa 120
atggaggata aaaaaattat acaattttgtg aatggaaagg aagacctgaa agttcagcac 180
agcagctaca gccagagggc tcagctattg aaggaccagc tcttcttggg gaaggctgcg 240
cttcagatca cagatgtgag attgcaggat gcaggggttt actgctgctt gatcggctat 300
ggcgggtgctg actacaagcg gattactttg aaagttcatg ccccgtagcg caacatcagc 360
caaagaattt ctgtggatcc tgtcacctct gaacatgaac taatgtgtca ggctgagggt 420
taccctgagg ctgaagtcac ctggacaagc agtgaccacc gagtcctgag tggcaaaacc 480
accatcacta attccaatag ggaagagaag cttttcaatg tgaccagcac gctgaacatc 540
aatgcaacag ctaatgagat tttctactgc acttttcaaa gatcagggtcc tgaggaaaac 600
aatactgccg agttgggtcat ccagaaacga ctgcccgttc cagcaagtga gaggactcat 660
ttcatgattc tgggaccttt cctgttgctt cttggtgtag tcctggcagt cactttctgt 720
ctaaaaaaac atggggagaat gatggatgtg gaaaaatgtt gcacccgaga taggaactca 780
aagaaacgaa atgatataca attgaagag acataa 816

<210> 8
<211> 271
<212> PRT
<213> Canis familiaris

<400> 8
Phe Thr Ile Thr Val Ser Lys Asp Leu Tyr Val Val Glu Tyr Gly Gly

eolf-seql.txt

```

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

```

<210> 9

eolf-seql.txt

<211> 660
<212> DNA
<213> Artificial Sequence

<220>
<223> codon optimized

<400> 9
 ttaccatca ccgtgtccaa ggacctgtac gtggctgagt acggcggcaa tgtgacctg 60
 gagtgcaagt tccccgtgga gaagcagctg aacctgttcg ccctcatcgt gtactgggag 120
 atggaggaca agaagatcat ccagttcgtg aacggcaagg aggacctgaa ggtgcagcac 180
 tccagctact cccagagagc ccagctgctg aaggaccagc tgttcctggg caaggccgcc 240
 ctgcagatca ccgacgtgag actgcaggac gccggcgtgt attgctgcct gatcggctac 300
 ggaggcgccg actacaagag gatcaccttg aaggtgcatg caccctacag gaacatcagc 360
 cagaggatca gcgtcgatcc cgtgaccagc gagcacgagc tgatgtgcca agccgagggc 420
 tatcccgagg ccgaagtgat ctggaccagc agcgaccaca gggtcctgag cggcaagacc 480
 accatcacca acagcaacag ggaggagaag ctgttcaacg tgaccagcac cctcaacatc 540
 aacgccaccg ccaacgagat cttctactgc accttccaga ggagcggccc cgaagagaac 600
 aacaccgccc agctgggtgat ccccagaga ctgcctgtgc ctgccagcga gaggaccac 660

<210> 10
<211> 220
<212> PRT
<213> Canis familiaris

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

eolf-seql.txt

Thr Ser Glu His Glu Leu Met Cys Gln Ala Glu Gly Tyr Pro Glu Ala
130 135 140

Glu Val Ile Trp Thr Ser Ser Asp His Arg Val Leu Ser Gly Lys Thr
145 150 155 160

Thr Ile Thr Asn Ser Asn Arg Glu Glu Lys Leu Phe Asn Val Thr Ser
165 170 175

Thr Leu Asn Ile Asn Ala Thr Ala Asn Glu Ile Phe Tyr Cys Thr Phe
180 185 190

Gln Arg Ser Gly Pro Glu Glu Asn Asn Thr Ala Glu Leu Val Ile Pro
195 200 205

Glu Arg Leu Pro Val Pro Ala Ser Glu Arg Thr His
210 215 220

<210> 11
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> codon optimized

<400> 11
tttaccatca ccgtgtccaa ggacctgtac gtggctgagt acggcggcaa tgtgaccatg 60
gagtgcgaagt tccccgtgga gaagcagctg aacctgttcg ccctcatcgt gtactgggag 120
atggaggaca agaagatcat ccagttcgtg aacggcaagg aggacctgaa ggtgcagcac 180
tccagctact cccagagagc ccagctgctg aaggaccagc tgttcctggg caaggccgcc 240
ctgcagatca ccgacgtgag actgcaggac gccggcgtgt attgctgcct gatcggctac 300
ggaggcgccg actacaagag gatcacctg aaggtgcatg caccctacag gaacatcagc 360
cagaggatca gcgtcgatcc cgtgaccagc gagcacgagc tgatgtgcca agccgagggc 420
tatcccaggg ccgaagtgat ctggaccagc agcgaccaca gggtcctgag cggcaagacc 480
accatcacca acagcaacag ggaggagaag ctgttcaacg tgaccagcac cctcaacatc 540
aacgccaccg ccaacgagat cttctactgc accttccaga ggagcggccc cgaagagaac 600
aacaccgccg agctggtgat ccccagaga ctgcctgtgc ctgccagcga gaggaccac 660
ggtaccgaca aaactcacac atgccaccg tgcccagcac ctgaactcct ggggggaccg 720
tcagtcttcc tcttcccccc aaaaccaag gacaccctca tgatctcccg gaccctgag 780
gtcacatgcg tgggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac 840
gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc 900
acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag 960
tacaagtgca aggtctccaa caaagccctc ccagccccc tgcagaaaac catctccaaa 1020

eolf-seql.txt

```

gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggatgagctg      1080
accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc      1140
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgctgctg      1200
gactccgacg gtccttctt cctctacagc aagctcaccg tggacaagag caggtggcag      1260
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag      1320
aagagcctct ccctgtctcc gggtaaata      1350

```

```

<210> 12
<211> 449
<212> PRT
<213> Canis familiaris
<400> 12

```

```

Phe Thr Ile Thr Val Ser Lys Asp Leu Tyr Val Val Glu Tyr Gly Gly
1          5          10          15

```

```

Asn Val Thr Met Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asn Leu
          20          25          30

```

```

Phe Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Lys Ile Ile Gln
          35          40          45

```

```

Phe Val Asn Gly Lys Glu Asp Leu Lys Val Gln His Ser Ser Tyr Ser
50          55          60

```

```

Gln Arg Ala Gln Leu Leu Lys Asp Gln Leu Phe Leu Gly Lys Ala Ala
65          70          75          80

```

```

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

```

```

Leu Ile Gly Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Leu Lys Val
100          105          110

```

```

His Ala Pro Tyr Arg Asn Ile Ser Gln Arg Ile Ser Val Asp Pro Val
115          120          125

```

```

Thr Ser Glu His Glu Leu Met Cys Gln Ala Glu Gly Tyr Pro Glu Ala
130          135          140

```

```

Glu Val Ile Trp Thr Ser Ser Asp His Arg Val Leu Ser Gly Lys Thr
145          150          155          160

```

```

Thr Ile Thr Asn Ser Asn Arg Glu Glu Lys Leu Phe Asn Val Thr Ser
165          170          175

```

```

Thr Leu Asn Ile Asn Ala Thr Ala Asn Glu Ile Phe Tyr Cys Thr Phe
180          185          190

```

eolf-seql.txt

Gln Arg Ser Gly Pro Glu Glu Asn Asn Thr Ala Glu Leu Val Ile Pro
195 200 205

Glu Arg Leu Pro Val Pro Ala Ser Glu Arg Thr His Gly Thr Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

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

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

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

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

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

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

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

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

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

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

eolf-seql.txt

<210> 13
 <211> 15
 <212> DNA
 <213> Mus musculus

<400> 13
 agttattatc tgtac

15

<210> 14
 <211> 5
 <212> PRT
 <213> Mus musculus

<400> 14

Ser Tyr Tyr Leu Tyr
 1 5

<210> 15
 <211> 48
 <212> DNA
 <213> Mus musculus

<400> 15
 ggggttaatc ctagtaatgg tggactaac ttcagtgaga agttcaag

48

<210> 16
 <211> 16
 <212> PRT
 <213> Mus musculus

<400> 16

Gly Val Asn Pro Ser Asn Gly Gly Thr Asn Phe Ser Glu Lys Phe Lys
 1 5 10 15

<210> 17
 <211> 33
 <212> DNA
 <213> Mus musculus

<400> 17
 agggattcta actacgacgg gggctttgac tac

33

<210> 18
 <211> 11
 <212> PRT
 <213> Mus musculus

<400> 18

Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr
 1 5 10

<210> 19
 <211> 45
 <212> DNA
 <213> Mus musculus

<400> 19
 agggccagca aaagtgtcag tacatctggc tttagttatt tgcac

45

eolf-seql.txt

<210> 20
 <211> 15
 <212> PRT
 <213> Mus musculus

<400> 20

Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His
 1 5 10 15

<210> 21
 <211> 21
 <212> DNA
 <213> Mus musculus

<400> 21

cttgcaccca acctagagtc t

21

<210> 22
 <211> 7
 <212> PRT
 <213> Mus musculus

<400> 22

Leu Ala Ser Asn Leu Glu Ser
 1 5

<210> 23
 <211> 27
 <212> DNA
 <213> Mus musculus

<400> 23

cagcacagtt gggagcttcc gctcacg

27

<210> 24
 <211> 9
 <212> PRT
 <213> Mus musculus

<400> 24

Gln His Ser Trp Glu Leu Pro Leu Thr
 1 5

<210> 25
 <211> 1353
 <212> DNA
 <213> Artificial Sequence

<220>

<223> caninized mouse antibody

<400> 25

gaggtgcagc tggtgcagtc cggcggcgat ctggtgaagc ctggaggcag cgtgagactg 60

agctgcgtgg ccagcggcta caccttcacc agctactacc tgtactgggt gaggcaggct 120

cctggcaaag gactgcagtg gatcggcggc gtgaatccta gcaacggcgg caccaacttc 180

eolf-seql.txt

```

agcgagaagt tcaagagcag ggccaccctg agcgtggaca aggccaagaa caccgcctac      240
atgcagctga actccctgag ggccgaggac accgccgtgt actactgcac caggagggac      300
agcaactacg acggcggcctt cgactactgg ggacagggaa ccctgctgac cgtgtccagc      360
gcttccacaa cggctccgtc ggtgtttccc ctggcaccta gctgcgggtc gacctcgggt      420
agcacagtgg cgctggcgtg tttggtgtcg ggatactttc ccgagccggt aacggtgtca      480
tggaactcag ggtcacttac atcaggagtc catacttttc cgtccgtgct gcagtcaagc      540
ggcttgcatt cactgtcctc gatggtgacg gtgccttcgt cgaggtggcc cagcgaaacg      600
ttcacttgta acgtagtaca cccggcctcc aacacgaaag tcgataaacc ggtattcaat      660
gagtgcagat gtacagacac ccctccctgt ccggtaccgg aacccttgagg agggccgagc      720
gtcctcatct tccctcccaa gccaaaagac atcttgcgca ttacgaggac accagaagtc      780
acgtgcgtag tgcttgatct cggtagagaa gatcccgagg tccagatctc gtggtttgtg      840
gatggaaagg aggtccacac cgcaaagact cagtcgcgcg agcagcagtt caatggcacg      900
tatcgggtcg tgagcgtgct tcctatcgag catcaggact ggctcaccgg gaaggagttc      960
aaatgccggg tcaatcatat cgacctcccg tcaccaatcg agcggaccat ctcgaaggct     1020
agaggaaggg cgcacaaacc ttcgggtctat gtgcttcccc catcgcccaa agagctttcc     1080
tcgtcggata cgggtgtccat tacatgcttg attaaggact tctatcctcc tgatattgat     1140
gtggaatggc aatcaaacgg acagcaggag ccggaacgca agcaccgaat gacccaccgg     1200
caattggacg aagatggtag ctactttctc tactcaaagc tctcagtcga caaatcccga     1260
tggcagcagg gagatccctt cacttgcgcc gtgatgcacg agacactcca aaatcattac     1320
acggaccttt cgttgagcca ctcgcccgga aag                                     1353

```

<210> 26
 <211> 451
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 26

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

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

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

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

Lys Ser Arg Ala Thr Leu Ser Val Asp Lys Ala Lys Asn Thr Ala Tyr

```

65              70              75              80
Met  Gln  Leu  Asn  Ser  Leu  Arg  Ala  Glu  Asp  Thr  Ala  Val  Tyr  Tyr  Cys
      85              90              95

Thr  Arg  Arg  Asp  Ser  Asn  Tyr  Asp  Gly  Gly  Phe  Asp  Tyr  Trp  Gly  Gln
      100             105             110

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

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

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

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

Leu  Gln  Ser  Ser  Gly  Leu  His  Ser  Leu  Ser  Ser  Met  Val  Thr  Val  Pro
      180             185             190

Ser  Ser  Arg  Trp  Pro  Ser  Glu  Thr  Phe  Thr  Cys  Asn  Val  Val  His  Pro
      195             200             205

Ala  Ser  Asn  Thr  Lys  Val  Asp  Lys  Pro  Val  Phe  Asn  Glu  Cys  Arg  Cys
      210             215             220

Thr  Asp  Thr  Pro  Pro  Cys  Pro  Val  Pro  Glu  Pro  Leu  Gly  Gly  Pro  Ser
      225             230             235             240

Val  Leu  Ile  Phe  Pro  Pro  Lys  Pro  Lys  Asp  Ile  Leu  Arg  Ile  Thr  Arg
      245             250             255

Thr  Pro  Glu  Val  Thr  Cys  Val  Val  Leu  Asp  Leu  Gly  Arg  Glu  Asp  Pro
      260             265             270

Glu  Val  Gln  Ile  Ser  Trp  Phe  Val  Asp  Gly  Lys  Glu  Val  His  Thr  Ala
      275             280             285

Lys  Thr  Gln  Ser  Arg  Glu  Gln  Gln  Phe  Asn  Gly  Thr  Tyr  Arg  Val  Val
      290             295             300

Ser  Val  Leu  Pro  Ile  Glu  His  Gln  Asp  Trp  Leu  Thr  Gly  Lys  Glu  Phe
      305             310             315             320

Lys  Cys  Arg  Val  Asn  His  Ile  Asp  Leu  Pro  Ser  Pro  Ile  Glu  Arg  Thr
      325             330             335

Ile  Ser  Lys  Ala  Arg  Gly  Arg  Ala  His  Lys  Pro  Ser  Val  Tyr  Val  Leu

```

340

345

350

Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp Thr Val Ser Ile Thr
 355 360 365

Cys Leu Ile Lys Asp Phe Tyr Pro Pro Asp Ile Asp Val Glu Trp Gln
 370 375 380

Ser Asn Gly Gln Gln Glu Pro Glu Arg Lys His Arg Met Thr Pro Pro
 385 390 395 400

Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val
 405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asp Pro Phe Thr Cys Ala Val Met
 420 425 430

His Glu Thr Leu Gln Asn His Tyr Thr Asp Leu Ser Leu Ser His Ser
 435 440 445

Pro Gly Lys
 450

<210> 27
 <211> 1368
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 27
 gaggtgcagc tgggtgcagtc cggcggcgat ctggtgaagc ctggaggcag cgtgagactg 60
 agctgcgtgg ccagcggcta caccttcacc agctactacc tgtactgggt gaggcaggct 120
 cctggcaaag gactgcagtg gatcggcggc gtgaatccta gcaacggcgg caccaacttc 180
 agcgagaagt tcaagagcag ggccaccctg agcgtggaca aggccaagaa caccgcctac 240
 atgcagctga actccctgag ggccgaggac accgccgtgt actactgcac caggagggac 300
 agcaactacg acggcggcctt cgactactgg ggacagggaa ccttgctgac cgtgtccagc 360
 gcttccacaa ccgcgccatc agtctttccg ttggcccat catgcgggtc gacgagcgga 420
 tcgactgtgg ccctggcgtg cttggtgtcg ggatactttc ccgaaccgt cacggtcagc 480
 tggaactccg gatcgcttac gagcgggtgtg catacgttcc cctcgggtctt gcaatcatca 540
 gggctctact cgctgtcgag catggtaacg gtgccctcat cgaggtggcc ctccgaaacg 600
 ttcacatgta acgtagcaca tccagcctcc aaaaccaagg tggataaacc cgtgccgaaa 660
 agagagaatg ggcggtgccc tcgacccctt gattgcccga agtgtccggc tccggaaatg 720
 ctcggtggac cctcagtgtt tatcttcct ccgaagccca aggacactct gctgatcgcg 780
 cgactccag aagtaacatg tgtagtggtg gaccttgatc ccgaggaccc cgaagtccag 840

eolf-seql.txt

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atctcctggt ttgtagatgg gaaacagatg cagaccgcaa aaactcaacc cagagaggag      900
cagttcaacg gaacataccg agtggtatcc gtccttccga ttggccacca ggactgggtg      960
aaaggaagc agtttacgtg taaagtcaac aataaggcgt tgcctagccc tattgagcgg      1020
acgatttcga aagctagggg acaggcccac cagccatcgg tctatgtcct tccgccttcc      1080
cgcgaggagc tctcgaagaa tacagtgagc cttacatgcc tcattaagga tttcttcccg      1140
cctgatatcg acgtagagtg gcaatcaaac ggtcaacagg agccggaatc caagtataga      1200
accactccgc cccagcttga cgaggacgga tcatactttt tgtattcaaa actgtcggtg      1260
gataagagcc ggtggcagag aggtgacacc ttcattctgtg cggtgatgca cgaagcactc      1320
cataatcact acaccaaga gagcctctcg cattcccccg gaaagtga      1368

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<210> 28
 <211> 455
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 28

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

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

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

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

Lys Ser Arg Ala Thr Leu Ser Val Asp Lys Ala Lys Asn Thr Ala Tyr
 65 70 75 80

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

Thr Arg Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr Trp Gly Gln
 100 105 110

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

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

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

eolf-seql.txt

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

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro
180 185 190

Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro
195 200 205

Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg Glu Asn Gly
210 215 220

Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met
225 230 235 240

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

Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu
260 265 270

Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
275 280 285

Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
305 310 315 320

Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
325 330 335

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

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

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

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

Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
405 410 415

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
420 425 430

eolf-seql.txt

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

Leu Ser His Ser Pro Gly Lys
 450 455

<210> 29
 <211> 1353
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 29
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 cctggcaaag gactgcagtg gatcggcggc gtgaatccta gcaacggcgg caccaacttc 180
 agcgagaagt tcaagagcag ggccaccctg agcgtggaca aggccaagaa caccgcctac 240
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 agcaactacg acggcggcctt cgactactgg ggacagggaa ccctgctgac cgtgtccagc 360
 gcttcaacca cagcgccgag cgtgttcctt ctggcgccgt cgtgcggttc cacctcggga 420
 tcaacagtgg ccctcgcttg tctcgtgagc ggatactttc cggagcctgt cagggtgtcg 480
 tggaatagcg gatcactcac gtcgggcgtg catacttttc catccgtctt gcaatcgagc 540
 ggattgtact cactctctc aaccgtcact gtcccctcgt cgcgctggcc ctcggagact 600
 ttacgtgca atgtagtcca tccggcgagc aacacgaagg tcgacaagcc cgtacccaag 660
 gaatcaacat gcaagtgcac ctcgccctgt cccgtccccg aatcccttgg tggcccctca 720
 gtgtttatct tccctccgaa gcctaaagac atcttgagaa tcacaagaac accggaaatc 780
 acgtgtgtgg tccttgactt gggacgcgag gaccctgagg tacaaatctc gtggttttgtg 840
 gacgggaaag aggtgcacac agcaaagaca caaccacgcg agcagcagtt taactcaacg 900
 tacagggtag tatccgtact tcccattgaa caccaggatt ggctcaccgg taaagaattc 960
 aaatgccgag tgaatcacat cgggcttcct agcccaattg agcggacgat ttccaaagct 1020
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 tcgtcagata cggtaacgtt gacgtgtctg atcaaagatt tctttcctcc cgaaattgat 1140
 gtggaatggc aaagcaatgg gcagcccgag ccgagtgcaa agtaccatac tactgcacca 1200
 cagctggacg aagatggatc gtatttcctc tactcgaaac tgtccgtgga taagtcccg 1260
 tggcagcaag gggacacctt cacttgcgcg gtcatgcacg aggcacttca gaaccactat 1320
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<210> 30
 <211> 451
 <212> PRT

<213> Artificial Sequence

<220>

<223> caninized mouse antibody

<400> 30

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

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

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

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

Lys Ser Arg Ala Thr Leu Ser Val Asp Lys Ala Lys Asn Thr Ala Tyr
 65 70 75 80

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

Thr Arg Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr Trp Gly Gln
 100 105 110

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

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

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

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

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

Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Val His Pro
 195 200 205

Ala Ser Asn Thr Lys Val Asp Lys Pro Val Pro Lys Glu Ser Thr Cys
 210 215 220

Lys Cys Ile Ser Pro Cys Pro Val Pro Glu Ser Leu Gly Gly Pro Ser
 225 230 235 240

Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Ile Leu Arg Ile Thr Arg

eolf-seql.txt

245

250

255

Thr Pro Glu Ile Thr Cys Val Val Leu Asp Leu Gly Arg Glu Asp Pro
 260 265 270

Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Glu Val His Thr Ala
 275 280 285

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

Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu Thr Gly Lys Glu Phe
 305 310 315 320

Lys Cys Arg Val Asn His Ile Gly Leu Pro Ser Pro Ile Glu Arg Thr
 325 330 335

Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser Val Tyr Val Leu
 340 345 350

Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp Thr Val Thr Leu Thr
 355 360 365

Cys Leu Ile Lys Asp Phe Phe Pro Pro Glu Ile Asp Val Glu Trp Gln
 370 375 380

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

Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val
 405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asp Thr Phe Thr Cys Ala Val Met
 420 425 430

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

Pro Gly Lys
 450

<210> 31
 <211> 666
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 31
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 atcagctgca gggcctccaa gagcgtgagc accagcggct tcagctacct gcactggtac 120

eolf-seql.txt

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aggcagaagc ccggacagag ccctcagctg ctgatcttcc tggccagcaa cctggagagc      180
ggcgtgcctg acagggttag cggaagcggc agcggcaccg acttcacact gaggatctcc      240
agggtggaag ccgacgacgc cggagtgtac tactgccagc acagctggga actgccccctg      300
accttcggcc agggcaccaa ggtggagatc aagaggaacg acgctcagcc agccgtgtac      360
ctcttcagc cttcgccgga ccagcttcat acgggggtcag cgtcgggtgt gtgcctgttg      420
aactcgtttt accccaagga cattaacgtg aagtggaagg tagacggggg aattcaagac      480
actggcattc aagagtccgt cacggaacaa gactcaaaag actcaacgta ttcactgtcg      540
tcaaccttga cgatgtcaag caccgagtat cttagccatg agctgtattc gtgcgagatc      600
accacaagt ccctcccctc cactcttatt aaatcctttc agcggtcgga atgtcagcgg      660
gtcgat

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<210> 32
 <211> 222
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 32

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

Glu Pro Ala Ser Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
 20 25 30

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

Gln Leu Leu Ile Phe Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
 50 55 60

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

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

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

Asn Asp Ala Gln Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln
 115 120 125

Leu His Thr Gly Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr
 130 135 140

Pro Lys Asp Ile Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp

145 150 155 160

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

Tyr Ser Leu Ser Ser Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser
 180 185 190

His Glu Leu Tyr Ser Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr
 195 200 205

Leu Ile Lys Ser Phe Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215 220

<210> 33
<211> 666
<212> DNA
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 33
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atcagctgca gggcctccaa gagcgtgagc accagcggct tcagctacct gcactggtac 120
aggcagaagc ccggacagcc tcctcagctg ctgatcttcc tggccagcaa cctggagagc 180
ggcgtgcctg acagggttag cggaagcggc agcggcaccg acttcacact gaggatctcc 240
agggtggaag ccgacgacgc cggagtgtac tactgccagc acagctggga actgccctg 300
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ctcttccagc cttcgccgga ccagcttcat acggggctcag cgtcggtggt gtgcctgttg 420
aactcgtttt accccaagga cattaacgtg aagtggaagg tagacggggt aattcaagac 480
actggcattc aagagtccgt cacggaacaa gactcaaaag actcaacgta ttactgtcgc 540
tcaaccttga cgatgtcaag caccgagtat cttagccatg agctgtattc gtgcgagatc 600
accacaagt ccctcccctc cactcttattc aaatcctttc agcggtcgga atgtcagcgg 660
gtcgat 666

<210> 34
<211> 222
<212> PRT
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 34

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

Glu Pro Ala Ser Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
Page 23

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

Gln Leu Leu Ile Phe Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
 50 55 60

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

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

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

Asn Asp Ala Gln Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln
 115 120 125

Leu His Thr Gly Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr
 130 135 140

Pro Lys Asp Ile Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp
 145 150 155 160

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

Tyr Ser Leu Ser Ser Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser
 180 185 190

His Glu Leu Tyr Ser Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr
 195 200 205

Leu Ile Lys Ser Phe Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215 220

<210> 35
 <211> 867
 <212> DNA
 <213> Canis familiaris

<400> 35
 atggggagcc ggcgggggcc ctggccgctc gtctgggccc tgctgcagct gggctggtgg 60
 ccaggatggc tcctagactc ccctgacagg ccctggagcc cgctcacctt ctccccggcg 120
 cagctcacgg tgcaggaggg agagaacgcc acgttcacct gcagcctggc cgacatcccc 180
 gacagcttcg tgctcaactg gtaccgcctg agcccccgca accagacgga caagctggcc 240
 gccttccagg aggaccgcat cgagccgggc cgggacaggc gcttccgcgt catgcggtg 300
 cccaacgggc gggacttcca catgagcatc gtcgctgcgc gcctcaacga cagcggcatc 360

eolf-seql.txt

tacctgtgcg gggccatcta cctgcccccc aacacacaga tcaacgagag tccccgcgca 420
gagctctccg tgacggagag aaccctggag cccccacac agagccccag cccccaccc 480
agactcagcg gccagttgca ggggctggtc atcggcgta cgagcgtgct ggtgggtgct 540
ctgctactgc tgctgctgac ctgggtcctg gccgctgtct tccccagggc cacccgaggt 600
gcctgtgtgt gcgggagcga ggacgagcct ctgaaggagg gccccgatgc agcgcccgtc 660
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gcgccctgtg ccccgagca gaccgagtat gccaccatcg tcttcccggg caggccggcg 780
tccccgggcc gcagggcctc ggccagcagc ctgcaggagg cccagcctcc gagccccgag 840
gacggacccg gcctgtggcc cctctga 867

<210> 36
<211> 288
<212> PRT
<213> Canis familiaris

<400> 36

Met Gly Ser Arg Arg Gly Pro Trp Pro Leu Val Trp Ala Val Leu Gln
1 5 10 15

Leu Gly Trp Trp Pro Gly Trp Leu Leu Asp Ser Pro Asp Arg Pro Trp
20 25 30

Ser Pro Leu Thr Phe Ser Pro Ala Gln Leu Thr Val Gln Glu Gly Glu
35 40 45

Asn Ala Thr Phe Thr Cys Ser Leu Ala Asp Ile Pro Asp Ser Phe Val
50 55 60

Leu Asn Trp Tyr Arg Leu Ser Pro Arg Asn Gln Thr Asp Lys Leu Ala
65 70 75 80

Ala Phe Gln Glu Asp Arg Ile Glu Pro Gly Arg Asp Arg Arg Phe Arg
85 90 95

Val Met Arg Leu Pro Asn Gly Arg Asp Phe His Met Ser Ile Val Ala
100 105 110

Ala Arg Leu Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Tyr Leu
115 120 125

Pro Pro Asn Thr Gln Ile Asn Glu Ser Pro Arg Ala Glu Leu Ser Val
130 135 140

Thr Glu Arg Thr Leu Glu Pro Pro Thr Gln Ser Pro Ser Pro Pro Pro
145 150 155 160

Arg Leu Ser Gly Gln Leu Gln Gly Leu Val Ile Gly Val Thr Ser Val

eof-seql.txt

165

170

175

Leu Val Gly Val Leu Leu Leu Leu Leu Leu Thr Trp Val Leu Ala Ala
180 185 190

Val Phe Pro Arg Ala Thr Arg Gly Ala Cys Val Cys Gly Ser Glu Asp
195 200 205

Glu Pro Leu Lys Glu Gly Pro Asp Ala Ala Pro Val Phe Thr Leu Asp
210 215 220

Tyr Gly Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro
225 230 235 240

Ala Pro Cys Ala Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro
245 250 255

Gly Arg Pro Ala Ser Pro Gly Arg Arg Ala Ser Ala Ser Ser Leu Gln
260 265 270

Gly Ala Gln Pro Pro Ser Pro Glu Asp Gly Pro Gly Leu Trp Pro Leu
275 280 285

<210> 37
<211> 870
<212> DNA
<213> Canis familiaris

<400> 37
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aaattcccgg tggaaaaaca gttaaacttg tttgcactaa tcgtctactg ggaaatggag 180
gataaaaaaa ttatacaatt tgtgaatgga aaggaagacc tgaaagttca gcacagcagc 240
tacagccaga gggctcagct attgaaggac cagctcttct tggggaaggc tgcgcttcag 300
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gctgactaca agcggattac tttgaaagtt catgccccgt accgcaacat cagccaaaga 420
atttctgtgg atcctgtcac ctctgaacat gaactaatgt gtcaggctga gggttaccct 480
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gccgagttgg tcatcccaga acgactgcc gttccagcaa gtgagaggac tcatttcag 720
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aaacatggga gaatgatgga tgtggaaaaa tggtgcaccc gagataggaa ctcaaagaaa 840
cgaaatgata tacaatttga agagacataa 870

eolf-seql.txt

<210> 38
 <211> 289
 <212> PRT
 <213> Canis familiaris

<400> 38

Met Arg Met Phe Ser Val Phe Thr Phe Met Ala Tyr Cys His Leu Leu
 1 5 10 15

Lys Ala Phe Thr Ile Thr Val Ser Lys Asp Leu Tyr Val Val Glu Tyr
 20 25 30

Gly Gly Asn Val Thr Met Glu Cys Lys Phe Pro Val Glu Lys Gln Leu
 35 40 45

Asn Leu Phe Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Lys Ile
 50 55 60

Ile Gln Phe Val Asn Gly Lys Glu Asp Leu Lys Val Gln His Ser Ser
 65 70 75 80

Tyr Ser Gln Arg Ala Gln Leu Leu Lys Asp Gln Leu Phe Leu Gly Lys
 85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Arg Leu Gln Asp Ala Gly Val Tyr
 100 105 110

Cys Cys Leu Ile Gly Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Leu
 115 120 125

Lys Val His Ala Pro Tyr Arg Asn Ile Ser Gln Arg Ile Ser Val Asp
 130 135 140

Pro Val Thr Ser Glu His Glu Leu Met Cys Gln Ala Glu Gly Tyr Pro
 145 150 155 160

Glu Ala Glu Val Ile Trp Thr Ser Ser Asp His Arg Val Leu Ser Gly
 165 170 175

Lys Thr Thr Ile Thr Asn Ser Asn Arg Glu Glu Lys Leu Phe Asn Val
 180 185 190

Thr Ser Thr Leu Asn Ile Asn Ala Thr Ala Asn Glu Ile Phe Tyr Cys
 195 200 205

Thr Phe Gln Arg Ser Gly Pro Glu Glu Asn Asn Thr Ala Glu Leu Val
 210 215 220

Ile Pro Glu Arg Leu Pro Val Pro Ala Ser Glu Arg Thr His Phe Met
 225 230 235 240

Ile Leu Gly Pro Phe Leu Leu Leu Leu Gly Val Val Leu Ala Val Thr
 Page 27

Phe Cys Leu Lys Lys His Gly Arg Met Met Asp Val Glu Lys Cys Cys
 260 265 270

Thr Arg Asp Arg Asn Ser Lys Lys Arg Asn Asp Ile Gln Phe Glu Glu
 275 280 285

Thr

<210> 39
 <211> 1410
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 39
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 tgcgtggcca gcggctacac cttcaccagc tactacctgt actgggtgag gcaggctcct 180
 ggcaaaggac tgcagtggat cggcggcgtg aatcctagca acggcggcac caacttcagc 240
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 aactacgacg gcggcttcga ctactgggga cagggaaacc tgctgaccgt gtccagcgct 420
 tccacaacgg ctccgtcggg gtttcccctg gcacctagct gcggggtcgac ctcgggtagc 480
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 aactcagggg cacttacatc aggagtccat acttttccgt ccgtgctgca gtcaagcggc 600
 ttgcattcac tgtcctcgat ggtgacggtg ctttcgtcga ggtggcccag cgaaacgttc 660
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 tgcagatgta cagacacccc tccctgtccg gtacccgaac cccttgaggg gccgagcgtc 780
 ctcatcttcc ctcccaagcc aaaagacatc ttgcgcatta cgaggacacc agaagtcacg 840
 tgcgtagtgc ttgatctcgg tagagaagat cccgaggtcc agatctcgtg gtttgtggat 900
 ggaaaggagg tccacaccgc aaagactcag tcgcgcgagc agcagttcaa tggcacgtat 960
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 tgccgggtca atcatatcga cctcccgtca ccaatcgagc ggaccatctc gaaggctaga 1080
 ggaagggcgc acaaaccttc ggtctatgtg cttcccccat cgcccaaaga gctttcctcg 1140
 tcggatacgg tgtccattac atgcttgatt aaggacttct atcctcctga tattgatgtg 1200
 gaatggcaat caaacggaca gcaggagccg gaacgcaagc accgaatgac cccaccgcaa 1260
 ttggacgaag atggtagcta ctttctctac tcaaagctct cagtcgacaa atcccgatgg 1320

eolf-seql.txt

cagcagggag atcccttcac ttgcgccgtg atgcacgaga cactccaaaa tcattacacg 1380
gacctttcgt tgagccactc gcccggaag 1410

<210> 40
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 40

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

Val Leu Ser Glu Val Gln Leu Val Gln Ser Gly Gly Asp Leu Val Lys
20 25 30

Pro Gly Gly Ser Val Arg Leu Ser Cys Val Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Tyr Leu Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Gln Trp Ile Gly Gly Val Asn Pro Ser Asn Gly Gly Thr Asn Phe Ser
65 70 75 80

Glu Lys Phe Lys Ser Arg Ala Thr Leu Ser Val Asp Lys Ala Lys Asn
85 90 95

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

Tyr Tyr Cys Thr Arg Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr
115 120 125

Trp Gly Gln Gly Thr Leu Leu Thr Val Ser Ser Ala Ser Thr Thr Ala
130 135 140

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

Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val
165 170 175

Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe
180 185 190

Pro Ser Val Leu Gln Ser Ser Gly Leu His Ser Leu Ser Ser Met Val
195 200 205

Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val

eolf-seql.txt

210

215

220

Val His Pro Ala Ser Asn Thr Lys Val Asp Lys Pro Val Phe Asn Glu
 225 230 235 240
 Cys Arg Cys Thr Asp Thr Pro Pro Cys Pro Val Pro Glu Pro Leu Gly
 245 250 255
 Gly Pro Ser Val Leu Ile Phe Pro Pro Lys Pro Lys Asp Ile Leu Arg
 260 265 270
 Ile Thr Arg Thr Pro Glu Val Thr Cys Val Val Leu Asp Leu Gly Arg
 275 280 285
 Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Glu Val
 290 295 300
 His Thr Ala Lys Thr Gln Ser Arg Glu Gln Gln Phe Asn Gly Thr Tyr
 305 310 315 320
 Arg Val Val Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu Thr Gly
 325 330 335
 Lys Glu Phe Lys Cys Arg Val Asn His Ile Asp Leu Pro Ser Pro Ile
 340 345 350
 Glu Arg Thr Ile Ser Lys Ala Arg Gly Arg Ala His Lys Pro Ser Val
 355 360 365
 Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp Thr Val
 370 375 380
 Ser Ile Thr Cys Leu Ile Lys Asp Phe Tyr Pro Pro Asp Ile Asp Val
 385 390 395 400
 Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Arg Lys His Arg Met
 405 410 415
 Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 420 425 430
 Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Pro Phe Thr Cys
 435 440 445
 Ala Val Met His Glu Thr Leu Gln Asn His Tyr Thr Asp Leu Ser Leu
 450 455 460
 Ser His Ser Pro Gly Lys
 465 470

<210> 41

eolf-seql.txt

<211> 1425
<212> DNA
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 41
atggccgtgc tggggctgct cttctgcctg gtgacattcc caagctgtgt gctaagcgag 60
gtgcagctgg tgcagtccgg cggcgatctg gtgaagcctg gaggcagcgt gagactgagc 120
tgcgtggcca gcggctacac cttcaccagc tactacctgt actgggtgag gcaggctcct 180
ggcaaaggac tgcagtggat cggcggcgtg aatcctagca acggcggcac caacttcagc 240
gagaagttca agagcagggc caccctgagc gtggacaagg ccaagaacac cgcctacatg 300
cagctgaact ccctgagggc cgaggacacc gccgtgtact actgcaccag gagggacagc 360
aactacgacg gcggcttcga ctactgggga cagggaaccc tgctgaccgt gtccagcgct 420
tccacaaccg cgccatcagt ctttccgttg gccccatcat gcgggtcgac gagcggatcg 480
actgtggccc tggcgtgctt ggtgtcggga tactttcccg aaccgcgtcac ggtcagctgg 540
aactccggat cgcttacgag cgggtgtgcat acgttcccct cgggtcttgca atcatcaggg 600
ctctactcgc tgtcgagcat ggtaacggtg ccctcatcga ggtggccctc cgaaacgttc 660
acatgtaacg tagcacatcc agcctccaaa accaaggtgg ataaaccgt gccgaaaaga 720
gagaatgggc ggggtgcctcg accccctgat tgccccaagt gtccgggtcc ggaaatgctc 780
ggtggaccct cagtgtttat cttccctccg aagcccaagg acactctgct gatcgcgcg 840
actccagaag taacatgtgt agtgggtggac cttgatcccg aggaccccga agtccagatc 900
tcctggtttg tagatgggaa acagatgcag accgcaaaaa ctcaaccag agaggagcag 960
ttcaacggaa cataccgagt ggtatccgtc cttccgattg gccaccagga ctggttgaaa 1020
gggaagcagt ttacgtgtaa agtcaacaat aaggcgttgc ctagccctat tgagcggacg 1080
atttcgaaag ctaggggaca ggcccaccag ccatcggctt atgtccttcc gccttcccgc 1140
gaggagctct cgaagaatac agtgagcctt acatgcctca ttaaggattt cttcccgctt 1200
gatatcgacg tagagtggca atcaaacggt caacaggagc cggaatccaa gtatagaacc 1260
actccgcccc agcttgacga ggacggatca tactttttgt attcaaaact gtcggtggat 1320
aagagccggt ggcagagagg tgacaccttc atctgtgcgg tgatgcacga agcactccat 1380
aatcactaca cccaagagag cctctcgcat tccccggaa agtga 1425

<210> 42
<211> 474
<212> PRT
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 42

Met Ala Val Leu Gly Leu Leu Phe Cys Leu Val Thr Phe Pro Ser Cys

eolf-seql.txt

```

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

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eolf-seql.txt

275

280

285

Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
290 295 300

Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
305 310 315 320

Phe Asn Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
325 330 335

Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
340 345 350

Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala
355 360 365

His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
370 375 380

Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
385 390 395 400

Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
405 410 415

Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
420 425 430

Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
435 440 445

Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
450 455 460

Gln Glu Ser Leu Ser His Ser Pro Gly Lys
465 470

<210> 43
<211> 1410
<212> DNA
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 43
atggccgtgc tggggctgct cttctgcctg gtgacattcc caagctgtgt gctaagcgag 60
gtgcagctgg tgcagtccgg cggcgatctg gtgaagcctg gaggcagcgt gagactgagc 120
tgcgtggcca gcggctacac cttcaccagc tactacctgt actgggtgag gcaggctcct 180
ggcaaaggac tgcagtggat cggcggcgtg aatcctagca acggcggcac caacttcagc 240

eolf-seql.txt

gagaagttca agagcagggc caccctgagc gtggacaagg ccaagaacac cgcctacatg 300
cagctgaact ccctgagggc cgaggacacc gccgtgtact actgcaccag gagggacagc 360
aactacgacg gcggcttcga ctactgggga cagggaaacc tgctgaccgt gtccagcgct 420
tcaaccacag cgccgagcgt gttccctctg gcgccgtcgt gcggttccac ctcgggatca 480
acagtggccc tcgcctgtct cgtgagcgga tactttccgg agcctgtcac ggtgtcgtgg 540
aatagcggat cactcacgtc gggcgtgcat acttttccat ccgtcttgca atcgagcgga 600
ttgtactcac tctcctcaac cgtcactgtc ccctcgtcgc gctggccctc ggagactttt 660
acgtgcaatg tagtccatcc ggcgagcaac acgaaggctc acaagcccgt acccaaggaa 720
tcaacatgca agtgcattct gccctgtccc gtccccgaat cccttggtgg cccctcagtg 780
tttatcttcc ctccgaagcc taaagacatc ttgagaatca caagaacacc ggaaatcacg 840
tgtgtggtcc ttgacttggg acgcgaggac cctgaggtac aaatctcgtg gtttgtggac 900
gggaaagagg tgcacacagc aaagacacaa ccacgcgagc agcagtttaa ctcaacgtac 960
agggtagtat ccgtacttcc cattgaacac caggattggc tcaccggtaa agaattcaaa 1020
tgccgagtga atcacatcgg gcttcctagc ccaattgagc ggacgatttc caaagctagg 1080
ggtcaggccc accagccgag cgtatacgtg ttgccgccct ccccgaagga gctgtcatcg 1140
tcagatacgg taacgttgac gtgtctgac aaagatttct ttcctcccga aattgatgtg 1200
gaatggcaaa gcaatgggca gcccagccc gagtcaaagt accatactac tgcaccacag 1260
ctggacgaag atggatcgta tttcctctac tcgaaactgt ccgtggataa gtcccggtagg 1320
cagcaagggg acaccttcac ttgcgcggtc atgcacgagg cacttcagaa ccactatacg 1380
gacttgagcc tctcgcatc gccaggggaag 1410

<210> 44

<211> 470

<212> PRT

<213> Artificial Sequence

<220>

<223> caninized mouse antibody

<400> 44

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

Val Leu Ser Glu Val Gln Leu Val Gln Ser Gly Gly Asp Leu Val Lys
20 25 30

Pro Gly Gly Ser Val Arg Leu Ser Cys Val Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Tyr Leu Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Gln Trp Ile Gly Gly Val Asn Pro Ser Asn Gly Gly Thr Asn Phe Ser
Page 34

eolf-seql.txt

```

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

```

340

345

350

Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser Val
 355 360 365

Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp Thr Val
 370 375 380

Thr Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Glu Ile Asp Val
 385 390 395 400

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

Thr Ala Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 420 425 430

Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Thr Phe Thr Cys
 435 440 445

Ala Val Met His Glu Ala Leu Gln Asn His Tyr Thr Asp Leu Ser Leu
 450 455 460

Ser His Ser Pro Gly Lys
 465 470

<210> 45

<211> 732

<212> DNA

<213> Artificial Sequence

<220>

<223> caninized mouse antibody

<400> 45

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cgctgcgata tcgtcctgac ccagaccct ctgagcctgt ccgtgagccc tggagaaccc	120
gccagcatca gctgcagggc ctccaagagc gtgagcacca gcggttcag ctacctgcac	180
tggtacaggc agaagcccg acagagccct cagctgctga tcttcctggc cagcaacctg	240
gagagcggcg tgcctgacag gtttagcgga agcggcagcg gcaccgactt cactactgagg	300
atctccaggg tggaagccga cgacgccgga gtgtactact gccagcacag ctgggaactg	360
cccctgacct tcggccaggg caccaaggtg gagatcaaga ggaacgacgc tcagccagcc	420
gtgtacctct tccagccttc gccggaccag cttcatagcg ggtcagcgtc ggtggtgtgc	480
ctgttgaact cgttttacct caaggacatt aacgtgaagt ggaaggtaga cggggtaatt	540
caagacactg gcattcaaga gtccgtcacg gaacaagact caaaagactc aacgtattca	600
ctgtcgtcaa cttgacgat gtcaagcacc gagtatctta gccatgagct gtattcgtgc	660
gagatcacc acaagtccct cccctccact cttatcaaat ctttcagcg gtcggaatgt	720

cagcgggtcg at

732

<210> 46
 <211> 244
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> caninized mouse antibody

<400> 46

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15

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

Leu Ser Val Ser Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ala Ser
 35 40 45

Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His Trp Tyr Arg Gln
 50 55 60

Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Phe Leu Ala Ser Asn Leu
 65 70 75 80

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

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

Tyr Cys Gln His Ser Trp Glu Leu Pro Leu Thr Phe Gly Gln Gly Thr
 115 120 125

Lys Val Glu Ile Lys Arg Asn Asp Ala Gln Pro Ala Val Tyr Leu Phe
 130 135 140

Gln Pro Ser Pro Asp Gln Leu His Thr Gly Ser Ala Ser Val Val Cys
 145 150 155 160

Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Val
 165 170 175

Asp Gly Val Ile Gln Asp Thr Gly Ile Gln Glu Ser Val Thr Glu Gln
 180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Met Ser
 195 200 205

Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser Cys Glu Ile Thr His
 210 215 220

eolf-seql.txt

Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe Gln Arg Ser Glu Cys
225 230 235 240

Gln Arg Val Asp

<210> 47
<211> 732
<212> DNA
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 47
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cgctgcgata tcgtcctgac ccagaccct cctagcctgt ccgtgagccc tggagaaccc 120
gccagcatca gctgcagggc ctccaagagc gtgagcacca gcggcttcag ctacctgcac 180
tggtacaggc agaagcccg acagcctcct cagctgctga tcttcctggc cagcaacctg 240
gagagcggcg tgcctgacag gtttagcgga agcggcagcg gcaccgactt cactctgagg 300
atctccaggg tggaagccga cgacgccgga gtgtactact gccagcacag ctgggaactg 360
cccctgacct tcggccaggg caccaaggtg gagatcaaga ggaacgacgc tcagccagcc 420
gtgtacctct tccagccttc gccggaccag cttcatagcg ggtcagcgtc ggtgggtgtgc 480
ctgttgaact cgttttaccc caaggacatt aacgtgaagt ggaaggtaga cggggtaatt 540
caagacactg gcattcaaga gtccgtcacg gaacaagact caaaagactc aacgtattca 600
ctgtcgtcaa cttgacgat gtcaagcacc gagtatctta gccatgagct gtattcgtgc 660
gagatcacc acaagtccct cccctccact cttatcaaat cctttcagcg gtcggaatgt 720
cagcgggtcg at 732

<210> 48
<211> 244
<212> PRT
<213> Artificial Sequence

<220>
<223> caninized mouse antibody

<400> 48

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

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

Leu Ser Val Ser Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ala Ser
35 40 45

Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His Trp Tyr Arg Gln
Page 38

eolf-seql.txt
60

50

55

Lys Pro Gly Gln Pro Pro Gln Leu Leu Ile Phe Leu Ala Ser Asn Leu
65 70 75 80

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

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

Tyr Cys Gln His Ser Trp Glu Leu Pro Leu Thr Phe Gly Gln Gly Thr
115 120 125

Lys Val Glu Ile Lys Arg Asn Asp Ala Gln Pro Ala Val Tyr Leu Phe
130 135 140

Gln Pro Ser Pro Asp Gln Leu His Thr Gly Ser Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Val
165 170 175

Asp Gly Val Ile Gln Asp Thr Gly Ile Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Met Ser
195 200 205

Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser Cys Glu Ile Thr His
210 215 220

Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe Gln Arg Ser Glu Cys
225 230 235 240

Gln Arg Val Asp

<210> 49
<211> 336
<212> PRT
<213> Canis familiaris

<400> 49

Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys
1 5 10 15

Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly
20 25 30

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

eolf-seql.txt

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

Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu
65 70 75 80

Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser Lys Thr Lys Val Asp
85 90 95

Lys Pro Val Pro Lys Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp
100 105 110

Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe
115 120 125

Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro
130 135 140

Glu Val Thr Cys Val Val Val Asp Leu Asp Pro Glu Asp Pro Glu Val
145 150 155 160

Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr
165 170 175

Gln Pro Arg Glu Glu Gln Phe Asn Gly Thr Tyr Arg Val Val Ser Val
180 185 190

Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys
195 200 205

Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser
210 215 220

Lys Ala Arg Gly Gln Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro
225 230 235 240

Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile
245 250 255

Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly
260 265 270

Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp
275 280 285

Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser
290 295 300

Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala Val Met His Glu Ala
305 310 315 320

eolf-seql.txt

Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
325 330 335

<210> 50

<211> 331

<212> PRT

<213> Artificial Sequence

<220>

<223> modified canine sequence

<400> 50

Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys
1 5 10 15

Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly
20 25 30

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

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

Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu
65 70 75 80

Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser Lys Thr Lys Val Asp
85 90 95

Lys Pro Val Phe Asn Glu Cys Arg Cys Thr Asp Thr Pro Pro Cys Pro
100 105 110

Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
115 120 125

Pro Lys Ala Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
130 135 140

Val Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
145 150 155 160

Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
165 170 175

Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
180 185 190

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

eolf-seql.txt

Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
210 215 220

Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu
225 230 235 240

Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
245 250 255

Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
260 265 270

Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
275 280 285

Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
290 295 300

Asp Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr
305 310 315 320

Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
325 330

<210> 51
<211> 331
<212> PRT
<213> Artificial Sequence

<220>
<223> modified canine sequence

<400> 51

Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys
1 5 10 15

Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly
20 25 30

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

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

Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu
65 70 75 80

Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser Lys Thr Lys Val Asp
85 90 95

Lys Pro Val Pro Lys Glu Ser Thr Cys Lys Cys Ile Ser Pro Cys Pro
100 105 110

eolf-seql.txt

Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
115 120 125

Pro Lys Ala Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
130 135 140

Val Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
145 150 155 160

Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
165 170 175

Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
180 185 190

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

Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
210 215 220

Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu
225 230 235 240

Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
245 250 255

Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
260 265 270

Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
275 280 285

Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
290 295 300

Asp Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr
305 310 315 320

Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
325 330

<210> 52
<211> 336
<212> PRT
<213> Artificial Sequence

<220>
<223> modified canine sequence

<400> 52

eofl-seql.txt

Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys
1 5 10 15

Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly
20 25 30

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

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

Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu
65 70 75 80

Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser Lys Thr Lys Val Asp
85 90 95

Lys Pro Val Pro Lys Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp
100 105 110

Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe
115 120 125

Ile Phe Pro Pro Lys Pro Lys Ala Thr Leu Leu Ile Ala Arg Thr Pro
130 135 140

Glu Val Thr Cys Val Val Val Asp Leu Asp Pro Glu Asp Pro Glu Val
145 150 155 160

Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr
165 170 175

Gln Pro Arg Glu Glu Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val
180 185 190

Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys
195 200 205

Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser
210 215 220

Lys Ala Arg Gly Gln Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro
225 230 235 240

Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile
245 250 255

Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly
260 265 270

eolf-seql.txt

Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp
275 280 285

Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser
290 295 300

Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala Val Met His Glu Ala
305 310 315 320

Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
325 330 335

<210> 53

<211> 401

<212> PRT

<213> Artificial Sequence

<220>

<223> canine and human

<400> 53

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Leu Asp Ser Pro Asp Arg
20 25 30

Pro Trp Ser Pro Leu Thr Phe Ser Pro Ala Gln Leu Thr Val Gln Glu
35 40 45

Gly Glu Asn Ala Thr Phe Thr Cys Ser Leu Ala Asp Ile Pro Asp Ser
50 55 60

Phe Val Leu Asn Trp Tyr Arg Leu Ser Pro Arg Asn Gln Thr Asp Lys
65 70 75 80

Leu Ala Ala Phe Gln Glu Asp Arg Ile Glu Pro Gly Arg Asp Arg Arg
85 90 95

Phe Arg Val Met Arg Leu Pro Asn Gly Arg Asp Phe His Met Ser Ile
100 105 110

Val Ala Ala Arg Leu Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile
115 120 125

Tyr Leu Pro Pro Asn Thr Gln Ile Asn Glu Ser Pro Arg Ala Glu Leu
130 135 140

Ser Val Thr Glu Arg Thr Leu Glu Pro Pro Thr Gln Ser Pro Ser Pro
145 150 155 160

Pro Pro Arg Leu Ser Gly Gln Leu Gln Gly Leu Val Gly Thr Asp Lys
165 170 175

eolf-seql.txt

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
180 185 190

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
195 200 205

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
210 215 220

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
225 230 235 240

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
245 250 255

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
260 265 270

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
275 280 285

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
290 295 300

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
305 310 315 320

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
325 330 335

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
340 345 350

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
355 360 365

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
370 375 380

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
385 390 395 400

Lys