



(86) Date de dépôt PCT/PCT Filing Date: 2005/11/10
(87) Date publication PCT/PCT Publication Date: 2006/05/18
(45) Date de délivrance/Issue Date: 2015/08/11
(85) Entrée phase nationale/National Entry: 2007/03/26
(86) N° demande PCT/PCT Application No.: EP 2005/055876
(87) N° publication PCT/PCT Publication No.: 2006/051091
(30) Priorités/Priorities: 2004/11/11 (EP04105684.7);
2004/11/11 (US60/627,773); 2004/11/30 (EP04106192.0);
2005/03/17 (EP05102117.8); 2005/08/08 (EP05107288.2)

(51) Cl.Int./Int.Cl. *A61K 39/42* (2006.01),
A61P 31/12 (2006.01)
(72) Inventeurs/Inventors:
TER MEULEN, JAN HENRIK, NL;
VAN DEN BRINK, EDWARD NORBERT, NL;
DE KRUIF, CORNELIS ADRIAAN, NL;
GOUDSMIT, JAAP, NL
(73) Propriétaire/Owner:
CRUCCELL HOLLAND B.V., NL
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : COMPOSITIONS CONTRE LE CORONAVIRUS DU SRAS ET UTILISATIONS DE CES COMPOSITIONS
(54) Title: COMPOSITIONS AGAINST SARS-CORONAVIRUS AND USES THEREOF

(57) **Abrégé/Abstract:**

The present invention provides compositions of binding molecules specifically binding to a coronavirus such as SARS-CoV and capable of neutralizing an infection caused by the virus. The compositions are suitable for diagnosing, preventing and/or treating a condition resulting from a coronavirus such as SARS-CoV.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 May 2006 (18.05.2006)

PCT

(10) International Publication Number
WO 2006/051091 A1

(51) International Patent Classification:

A61K 39/42 (2006.01) A61P 31/12 (2006.01)

(21) International Application Number:

PCT/EP2005/055876

(22) International Filing Date:

10 November 2005 (10.11.2005)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

04105684.7	11 November 2004 (11.11.2004)	EP
60/627,773	11 November 2004 (11.11.2004)	US
04106192.0	30 November 2004 (30.11.2004)	EP
05102117.8	17 March 2005 (17.03.2005)	EP
05107288.2	8 August 2005 (08.08.2005)	EP

(71) Applicant (for all designated States except US): **CRU-CELL HOLLAND B.V.** [NL/NL]; Archimedesweg 4, NL-2333 CN Leiden (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **TER MEULEN, Jan, Henrik** [DE/NL]; Leidse Kade 59, NL-1016 CX Amsterdam (NL). **VAN DEN BRINK, Edward Norbert** [NL/NL]; Burgemeester van Bruggenstraat 21, NL-1165 NS Halfweg (NL). **DE KRUIF, Cornelis Adriaan** [NL/NL]; Westerlaan 40, NL-3731 EL De Bilt (NL). **GOUDSMIT, Jaap** [NL/NL]; Koninginneweg 4, NL-1075 CX Amsterdam (NL).

(74) Agents: **KLEIN, Bart** et al.; Crucell Holland B.V., c/o Archimedesweg 4, NL-2333 CN Leiden (NL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report
- with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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TITLE OF THE INVENTION

Compositions against SARS-coronavirus and uses thereof

FIELD OF THE INVENTION

5 The invention relates to medicine. In particular the invention relates to compositions comprising binding molecules capable of specifically binding to and neutralizing SARS-coronavirus (SARS-CoV). The compositions are useful in the diagnosis of SARS-CoV and the prophylaxis and/or treatment of
10 a condition resulting from SARS-CoV.

BACKGROUND OF THE INVENTION

Recently a new and in several cases deadly clinical syndrome was observed in the human population, now called
15 severe acute respiratory syndrome (SARS) (Holmes, 2003). The syndrome is caused by a novel coronavirus (Ksiazek *et al.*, 2003), referred to as the SARS-CoV. The genome sequence of SARS-CoV has been determined (Rota *et al.*, 2003; Marra *et al.*, 2003). However, much remains to be learnt about this virus,
20 and means and methods for diagnostics, prophylaxis and/or treatment of the virus and the syndrome are needed. The present invention provides means and methods for use in diagnostics, prevention and/or treatment of SARS-CoV.

DESCRIPTION OF THE FIGURES

25 Figure 1 shows results from an ELISA, wherein the binding of the single-chain phage antibodies called SC03-014 and SC03-022 to an immobilized UV-inactivated SARS-CoV preparation (left column) or immobilized FBS (right column) was measured. The
30 binding of the control single-chain phage antibody called SC02-006 is also shown. On the y-axis the absorbance at 492 nm is shown.

Figure 2 shows an ELISA binding of IgGs CR03-014, CR03-022, control IgG and no IgG to an inactivated SARS-CoV preparation. On the Y-axis the absorbance at 492 nm is shown.

5

Figure 3 shows a FACS binding of the scFv phage antibodies SC03-014, SC03-022 and a control scFv phage antibody to the full length S protein expressed on HEK293T cells (left column) or mock transfected HEK293T cells (right column). On the Y-axis the mean fluorescence intensity is shown.

Figure 4 shows an ELISA binding of the antibodies CR03-014, CR03-022 and a control antibody to the S565 fragment (amino acids 1-565 of the S protein of SARS-CoV; left column), S318-510 fragment (amino acids 318-510 of the S protein of SARS-CoV; middle column) and an irrelevant control myc-tagged antigen (right column). On the Y-axis the absorbance at 492 nm is shown.

Figure 5 shows an ELISA binding of dilutions of antibodies CR03-014, CR03-022 and a control antibody to the S565 fragment of the S protein of SARS-CoV. On the Y-axis the absorbance at 492 nm and on the X-axis the amount of antibody in µg/ml is shown.

25

Figure 6 shows a competition ELISA for binding to the S565 fragment. Figure 6A shows competition between biotinylated antibody CR03-014 without competing antibody or with 1, 5 or 25 µg/ml competing antibody CR03-014, CR03-022 or control antibody. Figure 6B shows competition between biotinylated antibody CR03-022 with or without the competing antibodies as described above. On the Y-axis the % of maximal binding is

30

shown and on the X-axis the amount of the competing antibody in µg/ml.

Figure 7 shows a sandwich ELISA using anti-S protein antibodies. Immobilized antibodies CR03-014 and CR03-022 were used to capture S protein fragment S318-510. Bound fragment was detected using biotinylated antibody CR03-014, CR03-022 or control antibody. On the Y-axis the absorbance at 492 nm is shown.

Figure 8 shows binding of the monoclonal anti-SARS-antibodies CR03-014 and CR03-022 to the amino acid region of 318-510 of the S protein of the SARS-CoV strain Frankfurt 1 (called WT S318-510) and naturally occurring variants of the WT S318-510 fragment (variant A, mutation K344R; variant B, mutation S353F; variant C, mutation R426G and N437D; variant D, mutation Y436H; variant E, mutation Y442S; variant F, mutation N479S; variant G, mutation K344R, F360S, N479K and T487S; variant H, mutation K344R, F360S, L472P, D480G, and T487S; variant I, mutation K344R and F501Y). The control is an irrelevant myc-His tagged protein. On the Y-axis the absorbance at 492 nm is shown.

Figure 9 shows the comparison of the nucleotide and amino acid sequences of the SARS-CoV wild-type strain (SARS-CoV strain HKU 39849) and escape viruses of antibody CR03-014. Virus-infected cells were harvested 2 days post-infection and total RNA was isolated. cDNA was generated and used for DNA sequencing. Regions containing mutations are shown and the mutations are indicated in bold. Numbers above amino acids indicate amino acids numbers from S protein including signal

peptide. The sequences in Figure 9 are also represented by SEQ ID Nos:118-121.

Figure 10 shows binding of the monoclonal anti-SARS-antibodies CR03-014 and CR03-022 to the amino acid region of 318-510 of the S protein of the SARS-CoV strain Frankfurt 1 (called FRA1 S318-510) and an escape variant of antibody CR03-014 harboring a P462L substitution. On the Y-axis the absorbance at 492 nm is shown.

DESCRIPTION OF THE INVENTION

Herebelow follow definitions of terms as used in the invention

DEFINITIONS

Binding molecule

As used herein the term "binding molecule" refers to an intact immunoglobulin including monoclonal antibodies, such as chimeric, humanised or human monoclonal antibodies, or to an antigen-binding and/or variable domain comprising fragment of an immunoglobulin that competes with the intact immunoglobulin for specific binding to the binding partner of the immunoglobulin, e.g. the SARS-CoV. Regardless of structure, the antigen-binding fragment binds with the same antigen that is recognised by the intact immunoglobulin. An antigen-binding fragment can comprise a peptide or polypeptide comprising an amino acid sequence of at least 2 contiguous amino acid residues, at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 30 contiguous amino acid residues, at least 35 contiguous

amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least 200 contiguous amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of the binding molecule.

The term "binding molecule", as used herein includes all immunoglobulin classes and subclasses known in the art. Depending on the amino acid sequence of the constant domain of their heavy chains, binding molecules can be divided into the five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4.

Antigen-binding fragments include, *inter alia*, Fab, F(ab'), F(ab')₂, Fv, dAb, Fd, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage antibodies, diabodies, triabodies, tetrabodies, (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen binding to the (poly)peptide, etc. The above fragments may be produced synthetically or by enzymatic or chemical cleavage of intact immunoglobulins or they may be genetically engineered by recombinant DNA techniques. The methods of production are well known in the art and are described, for example, in Antibodies: A Laboratory Manual, Edited by: E. Harlow and D,

Lane (1988), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, which is incorporated herein by reference. A binding molecule or antigen-binding fragment thereof may have one or more binding sites. If there is more than one binding
5 site, the binding sites may be identical to one another or they may be different.

The binding molecule can be a naked or unconjugated binding molecule but can also be part of an immunoconjugate. A naked or unconjugated binding molecule is intended to refer to
10 a binding molecule that is not conjugated, operatively linked or otherwise physically or functionally associated with an effector moiety or tag, such as *inter alia* a toxic substance, a radioactive substance, a liposome, an enzyme. It will be understood that naked or unconjugated binding molecules do not
15 exclude binding molecules that have been stabilized, multimerized, humanized or in any other way manipulated, other than by the attachment of an effector moiety or tag. Accordingly, all post-translationally modified naked and unconjugated binding molecules are included herewith,
20 including where the modifications are made in the natural binding molecule-producing cell environment, by a recombinant binding molecule-producing cell, and are introduced by the hand of man after initial binding molecule preparation. Of course, the term naked or unconjugated binding molecule does
25 not exclude the ability of the binding molecule to form functional associations with effector cells and/or molecules after administration to the body, as some of such interactions are necessary in order to exert a biological effect.

30 Biological sample

As used herein, the term "biological sample" encompasses a variety of sample types, including blood and other liquid

samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures, or cells derived therefrom and the progeny thereof. The term also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides. The term encompasses various kinds of clinical samples obtained from any species, and also includes cells in culture, cell supernatants and cell lysates.

Complementarity determining regions (CDR)

The term "complementarity determining regions" as used herein means sequences within the variable regions of binding molecules, such as immunoglobulins, that usually contribute to a large extent to the antigen binding site which is complementary in shape and charge distribution to the epitope recognised on the antigen. The CDR regions can be specific for linear epitopes, discontinuous epitopes, or conformational epitopes of proteins or protein fragments, either as present on the protein in its native conformation or, in some cases, as present on the proteins as denatured, e.g., by solubilization in SDS. Epitopes may also consist of posttranslational modifications of proteins.

Expression-regulating nucleic acid sequence

The term "expression-regulating nucleic acid sequence" as used herein refers to polynucleotide sequences necessary for and/or affecting the expression of an operably linked coding sequence in a particular host organism. The expression-regulating nucleic acid sequences, such as *inter alia* appropriate transcription initiation, termination, promoter, enhancer sequences; repressor or activator sequences; efficient RNA

processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., ribosome binding sites); sequences that enhance protein stability; and when
5 desired, sequences that enhance protein secretion, can be any nucleic acid sequence showing activity in the host organism of choice and can be derived from genes encoding proteins, which are either homologous or heterologous to the host organism. The identification and employment of expression-regulating
10 sequences is routine to the person skilled in the art.

Functional variant

The term "functional variant", as used herein, refers to a binding molecule that comprises a nucleotide and/or amino acid
15 sequence that is altered by one or more nucleotides and/or amino acids compared to the nucleotide and/or amino acid sequences of the parent binding molecule and that is still capable of competing for binding to the binding partner, e.g. SARS-CoV, with the parent binding molecule. In other words,
20 the modifications in the amino acid and/or nucleotide sequence of the parent binding molecule do not significantly affect or alter the binding characteristics of the binding molecule encoded by the nucleotide sequence or containing the amino acid sequence, i.e. the binding molecule is still able to
25 recognize and bind its target. The functional variant may have conservative sequence modifications including nucleotide and amino acid substitutions, additions and deletions. These modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and random PCR-
30 mediated mutagenesis, and may comprise natural as well as non-natural nucleotides and amino acids.

Conservative amino acid substitutions include the ones in which the amino acid residue is replaced with an amino acid residue having similar structural or chemical properties. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cystine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). It will be clear to the skilled artisan that other classifications of amino acid residue families than the one used above can also be employed. Furthermore, a variant may have non-conservative amino acid substitutions, e.g., replacement of an amino acid with an amino acid residue having different structural or chemical properties. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing immunological activity may be found using computer programs well known in the art.

A mutation in a nucleotide sequence can be a single alteration made at a locus (a point mutation), such as transition or transversion mutations, or alternatively, multiple nucleotides may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleotide sequence. The mutations may be performed by any suitable method known in the art.

Host

The term "host", as used herein, is intended to refer to an organism or a cell into which a vector such as a cloning
5 vector or an expression vector has been introduced. The organism or cell can be prokaryotic or eukaryotic. It should be understood that this term is intended to refer not only to the particular subject organism or cell, but to the progeny of such an organism or cell as well. Because certain
10 modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent organism or cell, but are still included within the scope of the term "host" as used herein.

Human

The term "human", when applied to binding molecules as defined herein, refers to molecules that are either directly derived from a human or based upon a human sequence. When a binding
20 molecule is derived from or based on a human sequence and subsequently modified, it is still to be considered human as used throughout the specification. In other words, the term human, when applied to binding molecules is intended to include binding molecules having variable and constant regions
25 derived from human germline immunoglobulin sequences based on variable or constant regions either or not occurring in a human or human lymphocyte or in modified form. Thus, the human binding molecules may include amino acid residues not encoded by human germline immunoglobulin sequences, comprise
30 substitutions and/or deletions (e.g., mutations introduced by for instance random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). "Based on" as used herein refers

to the situation that a nucleic acid sequence may be exactly copied from a template, or with minor mutations, such as by error-prone PCR methods, or synthetically made matching the template exactly or with minor modifications. Semisynthetic
5 molecules based on human sequences are also considered to be human as used herein.

Isolated

The term "isolated", when applied to binding molecules as
10 defined herein, refers to binding molecules that are substantially free of other proteins or polypeptides, particularly free of other binding molecules having different antigenic specificities, and are also substantially free of other cellular material and/or chemicals. For example, when
15 the binding molecules are recombinantly produced, they are preferably substantially free of culture medium, and when the binding molecules are produced by chemical synthesis, they are preferably substantially free of chemical precursors or other chemicals, *i.e.*, they are separated from chemical precursors
20 or other chemicals which are involved in the synthesis of the protein. The term "isolated" when applied to nucleic acid molecules encoding binding molecules as defined herein, is intended to refer to nucleic acid molecules in which the nucleotide sequences encoding the binding molecules are free
25 of other nucleotide sequences, particularly nucleotide sequences encoding binding molecules that bind binding partners other than SARS-CoV. Furthermore, the term "isolated" refers to nucleic acid molecules that are substantially separated from other cellular components that naturally
30 accompany the native nucleic acid molecule in its natural host, *e.g.*, ribosomes, polymerases, or genomic sequences with which it is naturally associated. Moreover, "isolated" nucleic

acid molecules, such as a cDNA molecules, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically
5 synthesized.

Monoclonal antibody

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular
10 composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody displaying a single binding specificity which have variable and constant regions derived from or based on human
15 germline immunoglobulin sequences or derived from completely synthetic sequences. The method of preparing the monoclonal antibody is not relevant.

Nucleic acid molecule

20 The term "nucleic acid molecule" as used in the present invention refers to a polymeric form of nucleotides and includes both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide,
25 deoxynucleotide or a modified form of either type of nucleotide. The term also includes single- and double-stranded forms of DNA. In addition, a polynucleotide may include either or both naturally-occurring and modified nucleotides linked together by naturally-occurring and/or non-naturally occurring
30 nucleotide linkages. The nucleic acid molecules may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily

appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages
5 (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages
10 (e.g., alpha anomeric nucleic acids, etc.). The above term is also intended to include any topological conformation, including single-stranded, double-stranded, partially duplexed, triplex, hairpinned, circular and padlocked conformations. Also included are synthetic molecules that
15 mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. A reference to a
20 nucleic acid sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The complementary strand is also useful, e.g., for
25 antisense therapy, hybridization probes and PCR primers.

Operably linked

The term "operably linked" refers to two or more nucleic acid sequence elements that are usually physically linked and are
30 in a functional relationship with each other. For instance, a promoter is operably linked to a coding sequence if the promoter is able to initiate or regulate the transcription or

expression of a coding sequence, in which case the coding sequence should be understood as being "under the control of" the promoter.

5 Pharmaceutically acceptable excipient

By "pharmaceutically acceptable excipient" is meant any inert substance that is combined with an active molecule such as a drug, agent, or binding molecule for preparing an agreeable or convenient dosage form. The "pharmaceutically acceptable
10 excipient" is an excipient that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation comprising the drug, agent or binding molecule.

15 Specifically Binding

The term "specifically binding", as used herein, in reference to the interaction of a binding molecule, e.g. an antibody, and its binding partner, e.g. an antigen, means that the interaction is dependent upon the presence of a particular
20 structure, e.g. an antigenic determinant or epitope, on the binding partner. In other words, the antibody preferentially binds or recognizes the binding partner even when the binding partner is present in a mixture of other molecules or organisms. The binding may be mediated by covalent or non-
25 covalent interactions or a combination of both. In yet other words, the term "specifically binding" means immunospecifically binding to an antigen or a fragment thereof and not immunospecifically binding to other antigens. A binding molecule that immunospecifically binds to an antigen
30 may bind to other peptides or polypeptides with lower affinity as determined by, e.g., radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), BIACORE, or other assays known

in the art. Binding molecules or fragments thereof that immunospecifically bind to an antigen may be cross-reactive with related antigens. Preferably, binding molecules or fragments thereof that immunospecifically bind to an antigen
5 do not cross-react with other antigens.

Therapeutically effective amount

The term "therapeutically effective amount" refers to an amount of the binding molecule as defined herein that is
10 effective for preventing, ameliorating and/or treating a condition resulting from infection with SARS-CoV.

Treatment

The term "treatment" refers to therapeutic treatment as well
15 as prophylactic or preventative measures to cure or halt or at least retard disease progress. Those in need of treatment include those already inflicted with a condition resulting from infection with SARS-CoV as well as those in which infection with SARS-CoV is to be prevented. Subjects partially
20 or totally recovered from infection with SARS-CoV might also be in need of treatment. Prevention encompasses inhibiting or reducing the spread of SARS-CoV or inhibiting or reducing the onset, development or progression of one or more of the symptoms associated with infection with SARS-CoV.

25

Vector

The term "vector" denotes a nucleic acid molecule into which a second nucleic acid molecule can be inserted for introduction into a host where it will be replicated, and in some cases
30 expressed. In other words, a vector is capable of transporting a nucleic acid molecule to which it has been linked. Cloning as well as expression vectors are contemplated by the term

"vector", as used herein. Vectors include, but are not limited to, plasmids, cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC) and vectors derived from bacteriophages or plant or animal (including human) viruses. Vectors comprise an origin of replication recognised by the proposed host and in case of expression vectors, promoter and other regulatory regions recognised by the host. A vector containing a second nucleic acid molecule is introduced into a cell by transformation, transfection, or by making use of viral entry mechanisms. Certain vectors are capable of autonomous replication in a host into which they are introduced (e.g., vectors having a bacterial origin of replication can replicate in bacteria). Other vectors can be integrated into the genome of a host upon introduction into the host, and thereby are replicated along with the host genome.

SUMMARY OF THE INVENTION

The invention provides compositions of binding molecules capable of specifically binding to SARS-CoV and having SARS-CoV neutralizing activity. In a preferred embodiment, said binding molecules are human binding molecules. The invention further provides for the use of the compositions of the invention in the prophylaxis and/or treatment of a subject having, or at risk of developing, a condition resulting from SARS-CoV. Besides that, the invention pertains to the use of the compositions of the invention in the diagnosis/detection of SARS-CoV.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect the present invention encompasses compositions comprising at least two binding molecules. In an embodiment the binding molecules are immunoglobulins. Fragments of immunoglobulins still having the desired
5 functionality and/or activity of the complete immunoglobulin are also considered immunoglobulins according to the invention. Preferably, the at least two binding molecules, *i.e.* immunoglobulins, are capable of specifically binding to a coronavirus. Coronaviruses include, but not limited to, avian
10 infectious bronchitis virus, avian infectious laryngotracheitis virus, enteric coronavirus, equine coronavirus, coronavirus Group 1 species such as for instance human coronavirus 229E or human coronavirus NL63, coronavirus Group 2 species such as human coronavirus OC43 or chicken
15 enteric coronavirus, coronavirus Group 3 species, human enteric coronavirus 4408, and SARS-CoV. The compositions can be administered to a mammal to treat, prevent or ameliorate one or more symptoms associated with a coronavirus infection.

In an embodiment the invention relates to synergistic
20 compositions, *i.e.* compositions exhibiting synergistic coronavirus neutralizing activity. In other words, the compositions comprise at least two binding molecules, *i.e.* immunoglobulins, that are capable of specifically binding to a coronavirus and that have coronavirus neutralizing activity,
25 characterized in that the binding molecules act synergistically in neutralizing coronavirus. As used herein, the term "synergistic" means that the combined effect of the binding molecules when used in combination is greater than their additive effects when used individually. In other words,
30 the neutralizing activity of the composition is greater than the sum of the neutralizing activity of each immunoglobulin alone. In an embodiment none of the binding molecules, *i.e.*

immunoglobulins, present in the synergistic coronavirus neutralizing activity exhibiting compositions may have coronavirus neutralizing activity when used as an individual binding molecule. Alternatively, one binding molecule of the at least two binding molecules in the compositions exhibiting synergistic coronavirus neutralizing activity may have coronavirus neutralizing activity when used individually. In a preferred embodiment both of the at least two binding molecules, *i.e.* immunoglobulins, have coronavirus neutralizing activity when used individually. In an embodiment one of the at least two binding molecules in the synergistic coronavirus neutralizing activity exhibiting compositions may bind to a coronavirus and the other binding molecule may bind to a cell associated receptor of the coronavirus. Alternatively, both binding molecules may bind to either the coronavirus or cell associated receptor.

In a preferred embodiment of the invention the coronavirus is a SARS-CoV including animal or human SARS-CoV. Preferably, the SARS-CoV is a human SARS-CoV. In another aspect the invention thus provides compositions comprising at least two binding molecules, *i.e.* immunoglobulins, capable of specifically binding to SARS-CoV and preferably having SARS-CoV neutralizing activity. The compositions preferably exhibit synergistic SARS-CoV neutralizing activity. In other words, the compositions comprise at least two binding molecules, *i.e.* immunoglobulins, having SARS-CoV neutralizing activity, characterized in that the binding molecules act synergistically in neutralizing SARS-CoV. The SARS-CoV neutralizing activity of the composition is greater than the sum of the neutralizing activity of each immunoglobulin alone. In a preferred embodiment of the invention, the binding molecules in the compositions act synergistically in

neutralizing a plurality of SARS-CoV strains (see Table 1 for a list of several known SARS-CoV genome sequences and S protein genes). In another embodiment each of the immunoglobulins in the composition is capable of neutralizing a plurality of (different) SARS-CoV strains, preferably human SARS-CoV strains. In another embodiment at least one of the binding molecules, *i.e.* immunoglobulins, of the compositions of the invention is capable of neutralizing an animal SARS-CoV. The binding molecules in the compositions of the invention may neutralize coronavirus infectivity, such as SARS-CoV infectivity, by several modes of action including, but not limited to, preventing the attachment of the coronavirus to possible receptors on host cells, inhibition of the release of RNA into the cytoplasm of the cell, prevention of RNA transcription or translation, or inhibition or downregulation of coronavirus replication. Furthermore, the binding molecules may act by fixing complement or be capable of assisting in the lysis of enveloped coronavirus. They might also act as opsonins and augment phagocytosis of coronavirus either by promoting its uptake via Fc or C3b receptors or by agglutinating the coronavirus to make it more easily phagocytosed. The binding molecules in the compositions may have similar modes of action or may have different modes of action. In a specific embodiment, the compositions neutralize coronavirus, such as SARS-CoV, infectivity by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to infection of host cells by the coronavirus in the absence of said compositions. Assays for measuring virus neutralizing activity are known to the skilled person. Examples of such assays are described below.

The binding molecules, *i.e.* immunoglobulins, in the compositions of the invention may be capable of specifically binding to a coronavirus, such as SARS-CoV, in activated or inactivated/attenuated form. Methods for
5 inactivating/attenuating viruses are well known in the art and include, but are not limited to, heat inactivation, inactivation by UV irradiation, inactivation by gamma irradiation. The binding molecules may also be capable of specifically binding to one or more fragments of the
10 coronavirus including *inter alia* a preparation of one or more proteins and/or (poly)peptides derived from the coronavirus. Preferably, the fragment at least comprises an antigenic determinant recognised by the binding molecules of the invention. An "antigenic determinant" as used herein is a
15 moiety, such as a coronavirus (such as SARS-CoV), (poly)peptide, protein, glycoprotein, analog or fragment thereof, that is capable of binding to a binding molecule of the invention with sufficiently high affinity to form a detectable antigen-binding molecule complex.

20 In an embodiment the binding molecules, *i.e.* immunoglobulins, are capable of specifically binding to surface accessible proteins of a coronavirus which include, but are not limited to, inner and outer membrane proteins, proteins adhering to the cell wall, and potential secreted
25 proteins. Relevant proteins of SARS-CoV in that respect are *inter alia* the spike (S) protein, the membrane (matrix) protein, the (small) envelope protein, Orf 3, Orf 4, Orf 7, Orf 8, Orf 9, Orf 10 and Orf 14. The amino acid sequence of proteins and potential proteins of various known strains of
30 coronaviruses, such as SARS-CoV, can be found in the EMBL-database and/or other databases. For instance the complete genome of the SARS coronavirus Urbani can be found in the

EMBL-database under accession number AY278741, the complete genome of the SARS coronavirus HSR 1 can be found under accession number AY323977, the complete genome of the SARS coronavirus Frankfurt 1 can be found under accession number
5 AY291315 and the complete genome of the SARS coronavirus TOR2 can be found under accession number AY274119.

In an embodiment at least one of the binding molecules, *i.e.* immunoglobulins, in the compositions of the invention is capable of specifically binding to the S protein of SARS-CoV.
10 The other binding molecule may bind to a receptor of SARS-CoV present on or associated with target cells. An example of such a receptor is the ACE-2 receptor (see Li *et al.*, 2003). In another embodiment all binding molecules in the compositions of the invention are capable of specifically binding to the S
15 protein of SARS-CoV.

In yet another embodiment at least one of the binding molecules in the compositions of the invention is capable of specifically binding to the extracellular domain of the S protein of SARS-CoV. This domain consists of amino acids 15-
20 1195 of the S protein. In a specific embodiment at least one binding molecule in the compositions of the invention is capable of specifically binding to amino acids 318-510 of the S protein of SARS-CoV. The neutralizing binding molecules, *i.e.* immunoglobulins, in the compositions of the invention may
25 react with overlapping, competing epitopes, but preferably they react with different/distinct, non-competing epitopes of the coronavirus, such as SARS-CoV.

Another aspect of the invention are compositions comprising at least two binding molecules capable of
30 specifically binding to a coronavirus, such as SARS-CoV, wherein the binding molecules are capable of reacting with different, non-competing epitopes of the coronavirus.

Preferably, the coronavirus is a human coronavirus, more preferably the coronavirus is SARS-CoV. Compositions comprising at least two binding molecules wherein each binding molecule binds to a different epitope or site on a virus are more suitable for preventing the escape of resistant variants of the virus compared to compositions comprising at least two binding molecules wherein each binding molecule binds to an overlapping epitope or site on the virus.

In a specific embodiment the different, non-competing epitopes recognised by the binding molecules, *i.e.*

immunoglobulins, in the compositions of the invention are located on the S protein of SARS-CoV, particularly the extracellular domain of the S protein, more particularly within amino acids 318-510 of the S protein. In another

aspect, at least one of the binding molecules, *i.e.*

immunoglobulins, of the compositions of the invention is capable of reacting with amino acids 318-510 of the S protein of a human and an animal SARS-CoV. In another embodiment at least one of the binding molecules, *i.e.* immunoglobulins, of the compositions of the invention reacts with an epitope comprising the amino acid sequence of SEQ ID NO:128. The epitope may consist of 11, 11 to 15, 11 to 20, 11 to 25, 11 to 30, 11 to 35, 11 to 40, 11 to 45 or even more amino acids. In another aspect at least one of the binding molecules, *i.e.*

immunoglobulins, in the compositions of the invention is capable of reacting with amino acids 318-510 of the S protein of a SARS-CoV, wherein the amino acid at position 479 is an amino acid other than asparagine, to a similar extent as with amino acids 318-510 of the S protein of a SARS-CoV, wherein

the amino acid at position 479 is an asparagine. In other words, substitution of the amino acid at position 479 does not dramatically influence the binding of at least one of the

immunoglobulins in the compositions of the invention to amino acids 318-510 of the S protein of SARS-CoV. "To a similar extent" as defined above means that the binding molecule binds to amino acids 318-510 of the S protein of a SARS-CoV, wherein
5 the amino acid at position 479 is an amino acid other than asparagines, in an amount of at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, preferably at least 100%, compared to the binding of the binding molecule to amino acids 318-510 of the S protein of a
10 SARS-CoV, wherein the amino acid at position 479 is an asparagine. Binding can be measured by methods well known to a person skilled in the art such as for instance ELISA.

The binding molecules in the compositions according to the invention are preferably human binding molecules, *i.e.*
15 immunoglobulins. They can be intact immunoglobulin molecules such as polyclonal or monoclonal antibodies, in particular human monoclonal antibodies, or the binding molecules can be antigen-binding fragments including, but not limited to, Fab, F(ab'), F(ab')₂, Fv, dAb, Fd, complementarity determining
20 region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage antibodies, diabodies, triabodies, tetrabodies, and (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen
25 binding to the SARS-CoV or fragment thereof. The human binding molecules are preferably human monoclonal antibodies. The binding molecules in the compositions can be in non-isolated or isolated form. The compositions may further comprise at least one other therapeutic agent. Preferably, the therapeutic
30 agent is useful in the prophylaxis and/or treatment of a condition resulting from a coronavirus such as for instance SARS-CoV.

Typically, binding molecules can bind to their binding partners, *i.e.* a coronavirus or fragments thereof, with an affinity constant (K_d -value) that is lower than 0.2×10^{-4} M, 1.0×10^{-5} M, 1.0×10^{-6} M, 1.0×10^{-7} M, preferably lower than 1.0×10^{-8} M, more preferably lower than 1.0×10^{-9} M, more preferably lower than 1.0×10^{-10} M, even more preferably lower than 1.0×10^{-11} M, and in particular lower than 1.0×10^{-12} M. The affinity constants can vary for antibody isotypes. For example, affinity binding for an IgM isotype refers to a binding affinity of at least about 1.0×10^{-7} M. Affinity constants can for instance be measured using surface plasmon resonance, *i.e.* an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE* system (Pharmacia Biosensor AB, Uppsala, Sweden).

The binding molecules may bind to a coronavirus in soluble form such as for instance in a sample or may bind to a coronavirus bound or attached to a carrier or substrate, *e.g.*, microtiter plates, membranes and beads, etc. Carriers or substrates may be made of glass, plastic (*e.g.*, polystyrene), polysaccharides, nylon, nitrocellulose, or teflon, etc. The surface of such supports may be solid or porous and of any convenient shape. Furthermore, the binding molecules may bind to a coronavirus in purified/isolated or non-purified/non-isolated form.

In a preferred embodiment, the binding molecules, *i.e.* immunoglobulins, of the compositions according to the invention comprise at least a CDR3 region, preferably a heavy chain CDR3 region, comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2. In another embodiment the binding molecules comprising the heavy chain CDR3 region of SEQ ID

*Trade-mark

NO:1 further comprise a light chain CDR3 region comprising the amino acid sequence of SEQ ID NO:129. In another embodiment, the binding molecules comprising the heavy chain CDR3 region of SEQ ID NO:1 further comprise a heavy chain CDR1, heavy chain CDR2, light chain CDR1 and light chain CDR2 region comprising the amino acid sequence of SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, and SEQ ID NO:133, respectively. In another embodiment the binding molecules comprising the heavy chain CDR3 region of SEQ ID NO:2 further comprise a light chain CDR3 region comprising the amino acid sequence of SEQ ID NO:134. In another embodiment, the binding molecules comprising the heavy chain CDR3 region of SEQ ID NO:2 further comprise a heavy chain CDR1, heavy chain CDR2, light chain CDR1 and light chain CDR2 region comprising the amino acid sequence of SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138, respectively. In yet another embodiment, the binding molecules according to the invention comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In an embodiment the binding molecules, *i.e.* immunoglobulins, in the compositions of the invention comprise at least one CDR region of a binding molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In another embodiment they comprise two, three, four, five or even all six CDR regions.

In a further embodiment, the binding molecules according to the invention comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:8, or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10.

In a preferred embodiment the binding molecules having coronavirus, such as SARS-CoV, neutralizing activity are administered in IgG1 or IgA format.

In another aspect of the invention the compositions may
5 comprise at least one functional variant of a binding molecule as defined herein. The compositions may also consist of only functional variants of binding molecules as herein described. Molecules are considered to be functional variants of a binding molecule, if the variants are capable of competing for
10 specifically binding to a coronavirus, such as SARS-CoV, or a fragment thereof with the parent binding molecules. In other words, when the functional variants are still capable of binding to the coronavirus, such as SARS-CoV, or a fragment thereof. Preferably, the functional variants are capable of
15 neutralizing coronavirus, such as SARS-CoV, infectivity and should together with the other binding molecule (or other functional variant) or other binding molecules (or other functional variants) form a composition exhibiting synergistic coronavirus, such as SARS-CoV, neutralizing activity. The
20 neutralizing activity of a functional variant may either be higher or be lower compared to the parent binding molecules.

Functional variants include, but are not limited to, derivatives that are substantially similar in primary structural sequence, but which contain e.g. *in vitro* or *in*
25 *vivo* modifications, chemical and/or biochemical, that are not found in the parent binding molecule. Such modifications are well known to the skilled artisan and include *inter alia* acetylation, acylation, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid
30 derivative, glycosylation, methylation, pegylation, proteolytic processing, phosphorylation, and the like.

Alternatively, functional variants can be binding molecules as defined in the present invention comprising an amino acid sequence containing substitutions, insertions, deletions or combinations thereof of one or more amino acids compared to the amino acid sequences of the parent binding molecules. Furthermore, functional variants can comprise truncations of the amino acid sequence at either or both the amino or carboxy termini. Functional variants may have the same or different, either higher or lower, binding affinities compared to the parent binding molecule but are still capable of binding to a coronavirus, such as SARS-CoV, or a fragment thereof and preferably still capable of neutralizing coronavirus, such as SARS-CoV, infectivity. For instance, functional variants may have increased or decreased binding affinities for a coronavirus, such as SARS-CoV, or a fragment thereof compared to the parent binding molecules. Preferably, the amino acid sequences of the variable regions, including, but not limited to, framework regions, hypervariable regions, in particular the CDR3 regions, are modified. Generally, the light chain and the heavy chain variable regions comprise three hypervariable regions, comprising three CDRs, and more conserved regions, the so-called framework regions (FRs). The hypervariable regions comprise amino acid residues from CDRs and amino acid residues from hypervariable loops. Functional variants intended to fall within the scope of the present invention have at least about 50% to about 99%, preferably at least about 60% to about 99%, more preferably at least about 70% to about 99%, even more preferably at least about 80% to about 99%, most preferably at least about 90% to about 99%, in particular at least about 95% to about 99%, and in particular at least about 97% to about 99% amino acid sequence homology with the parent binding molecules as defined herein. Computer

algorithms such as *inter alia* Gap or Bestfit known to a person skilled in the art can be used to optimally align amino acid sequences to be compared and to define similar or identical amino acid residues. Functional variants can be obtained by
5 altering the parent binding molecules or parts thereof by general molecular biology methods known in the art including, but not limited to, error-prone PCR, oligonucleotide-directed mutagenesis and site-directed mutagenesis.

In yet a further aspect, the invention includes
10 compositions comprising at least one immunoconjugate, *i.e.* a molecule comprising at least one binding molecule or functional variant thereof as defined herein and further comprising at least one tag. Also contemplated in the present invention are compositions consisting of immunoconjugates. The
15 compositions may further comprise another molecule, such as a therapeutic agent or immunoconjugate having a different specificity. In a further embodiment, the immunoconjugates of the invention may comprise more than one tag. These tags can be the same or distinct from each other and can be
20 joined/conjugated non-covalently to the binding molecules. The tag(s) can also be joined/conjugated directly to the binding molecules through covalent bonding. Alternatively, the tag(s) can be joined/conjugated to the binding molecules by means of one or more linking compounds. Techniques for conjugating tags
25 to binding molecules are well known to the skilled artisan.

The tags of the immunoconjugates of the present invention may be therapeutic agents, but preferably they are detectable moieties/agents. Compositions comprising immunoconjugates comprising a detectable agent can be used diagnostically to,
30 for example, assess if a subject has been infected with a coronavirus, such as SARS-CoV, or monitor the development or progression of a coronavirus, such as SARS-CoV, infection as

part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. However, they may also be used for other detection and/or analytical and/or diagnostic purposes. Detectable moieties/agents include, but
5 are not limited to, enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The tags used to label the binding molecules for detection and/or analytical and/or
10 diagnostic purposes depend on the specific detection/analysis/diagnosis techniques and/or methods used such as *inter alia* immunohistochemical staining of (tissue) samples, flow cytometric detection, scanning laser cytometric detection, fluorescent immunoassays, enzyme-linked
15 immunosorbent assays (ELISA's), radioimmunoassays (RIA's), bioassays (e.g., neutralisation assays), Western blotting applications, etc. For immunohistochemical staining of tissue samples preferred labels are enzymes that catalyze production and local deposition of a detectable product. Furthermore, the
20 compositions of the invention can also be attached to solid supports, which are particularly useful for *in vitro* immunoassays or purification of a coronavirus or a fragment thereof. Such solid supports might be porous or nonporous, planar or nonplanar. The binding molecules of the present
25 invention or functional fragments thereof can be fused to marker sequences, such as a peptide to facilitate purification. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate. In another aspect the binding molecules of the invention may be
30 conjugated/attached to one or more antigens. Preferably, these antigens are antigens which are recognised by the immune

system of a subject to which the binding molecule-antigen conjugate is administered.

Next to producing immunoconjugates chemically by conjugating, directly or indirectly via for instance a linker, the immunoconjugates can be produced as fusion proteins comprising the binding molecules of the invention and a suitable tag. Fusion proteins can be produced by methods known in the art such as, e.g., recombinantly by constructing nucleic acid molecules comprising nucleotide sequences encoding the binding molecules in frame with nucleotide sequences encoding the suitable tag(s) and then expressing the nucleic acid molecules.

It is another aspect of the present invention to provide a nucleic acid molecule encoding at least a binding molecule or functional fragment thereof present in the compositions according to the invention. Such nucleic acid molecules can be used as intermediates for cloning purposes, e.g. in the process of affinity maturation described above. In a preferred embodiment, the nucleic acid molecules are isolated or purified.

The skilled man will appreciate that functional variants of these nucleic acid molecules are also intended to be a part of the present invention. Functional variants are nucleic acid sequences that can be directly translated, using the standard genetic code, to provide an amino acid sequence identical to that translated from the parent nucleic acid molecules.

Preferably, the nucleic acid molecules encode binding molecules comprising a CDR3 region, preferably a heavy chain CDR3 region, comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2. In another embodiment the nucleic acid molecules of the invention encode binding molecules comprising a light chain CDR3 region comprising the amino acid sequence

of SEQ ID NO:129 or SEQ ID NO:134. In yet another embodiment the nucleic acid molecules of the invention encode binding molecules comprising a heavy chain CDR1 region comprising the amino acid sequence of SEQ ID NO:130 or SEQ ID NO:135; a heavy chain CDR2 region comprising the amino acid sequence of SEQ ID NO:131 or SEQ ID NO:136; a light chain CDR1 region comprising the amino acid sequence of SEQ ID NO:132 or SEQ ID NO:137; and/or a light chain CDR2 region comprising the amino acid sequence of SEQ ID NO:133 or SEQ ID NO:138.

Even more preferably, the nucleic acid molecules encode binding molecules comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6.

In yet another embodiment, the nucleic acid molecules encode binding molecules comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:8 or they encode a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10.

In a specific embodiment of the invention the nucleic acid molecules encoding the heavy chain variable region of the binding molecules of the invention comprise the nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:5.

In yet another specific embodiment of the present invention, the nucleic acid molecules encoding the light chain variable region of the binding molecules of the invention comprise the nucleotide sequence of SEQ ID NO:7 or SEQ ID NO:9. The nucleic acid molecules of the invention may even contain the nucleotide sequences or parts thereof of the at

least two binding molecules present in the compositions of the invention.

It is another aspect of the invention to provide vectors, *i.e.* nucleic acid constructs, comprising one or more nucleic acid molecules according to the present invention. Vectors can be derived from plasmids such as *inter alia* F, R1, RP1, Col, pBR322, TOL, Ti, etc; cosmids; phages such as lambda, lambdoid, M13, Mu, P1, P22, Q β , T-even, T-odd, T2, T4, T7, etc; plant viruses; or animal viruses. Vectors can be used for cloning and/or for expression of the binding molecules of the invention and might even be used for gene therapy purposes. Vectors comprising one or more nucleic acid molecules according to the invention operably linked to one or more expression-regulating nucleic acid molecules are also covered by the present invention. The binding molecules present in the compositions of the invention may be expressed on separate vectors but may also be expressed on the same vector. The choice of the vector(s) is dependent on the recombinant procedures followed and the host used. Introduction of vectors in host cells can be effected by *inter alia* calcium phosphate transfection, virus infection, DEAE-dextran mediated transfection, lipofectamin transfection or electroporation. Vectors may be autonomously replicating or may replicate together with the chromosome into which they have been integrated. Preferably, the vectors contain one or more selection markers. The choice of the markers may depend on the host cells of choice, although this is not critical to the invention as is well known to persons skilled in the art. They include, but are not limited to, kanamycin, neomycin, puromycin, hygromycin, zeocin, thymidine kinase gene from Herpes simplex virus (HSV-TK), dihydrofolate reductase gene from mouse (dhfr). Vectors comprising one or more nucleic acid

molecules encoding the binding molecules as described above operably linked to one or more nucleic acid molecules encoding proteins or peptides that can be used to isolate the binding molecules are also covered by the invention. These proteins or peptides include, but are not limited to, glutathione-S-transferase, maltose binding protein, metal-binding polyhistidine, green fluorescent protein, luciferase and beta-galactosidase.

Hosts containing one or more copies of the vectors mentioned above are an additional subject of the present invention. Preferably, the hosts are host cells. Host cells include, but are not limited to, cells of mammalian, plant, insect, fungal or bacterial origin. Bacterial cells include, but are not limited to, cells from Gram positive bacteria such as several species of the genera *Bacillus*, *Streptomyces* and *Staphylococcus* or cells of Gram negative bacteria such as several species of the genera *Escherichia*, such as *E. coli*, and *Pseudomonas*. In the group of fungal cells preferably yeast cells are used. Expression in yeast can be achieved by using yeast strains such as *inter alia* *Pichia pastoris*, *Saccharomyces cerevisiae* and *Hansenula polymorpha*.

Furthermore, insect cells such as cells from *Drosophila* and Sf9 can be used as host cells. Besides that, the host cells can be plant cells expression systems using mammalian cells such as Chinese Hamster Ovary (CHO) cells, COS cells, BHK cells or Bowes melanoma cells are preferred in the present invention. Mammalian cells provide expressed proteins with posttranslational modifications that are most similar to natural molecules of mammalian origin. Since the present invention deals with molecules that may have to be administered to humans, a completely human expression system would be particularly preferred. Therefore, even more

preferably, the host cells are human cells. Examples of human cells are *inter alia* HeLa, 911, AT1080, A549, 293 and HEK293T cells. Preferred mammalian cells are human retina cells such as 911 cells or the cell line deposited at the European
5 Collection of Cell Cultures (ECACC), CAMR, Salisbury, Wiltshire SP4 OJG, Great Britain on 29 February 1996 under number 96022940 and marketed under the trademark PER.C6® (PER.C6 is a registered trademark of Crucell Holland B.V.). For the purposes of this application "PER.C6" refers to cells
10 deposited under number 96022940 or ancestors, passages upstream or downstream as well as descendants from ancestors of deposited cells, as well as derivatives of any of the foregoing. In preferred embodiments, the human producer cells comprise at least a functional part of a nucleic acid sequence
15 encoding an adenovirus E1 region in expressible format. In even more preferred embodiments, said host cells are derived from a human retina and immortalised with nucleic acids comprising adenoviral E1 sequences, such as the cell line deposited at the European Collection of Cell Cultures (ECACC),
20 CAMR, Salisbury, Wiltshire SP4 OJG, Great Britain on 29 February 1996 under number 96022940 and marketed under the trademark PER.C6®, and derivatives thereof. Production of recombinant proteins in host cells can be performed according to methods well known in the art. The use of the cells
25 marketed under the trademark PER.C6® as a production platform for proteins of interest has been described in WO 00/63403.

Methods of producing binding molecules or functional variants are well known to the skilled artisan. One method comprises the steps of a) culturing a host as defined above
30 under conditions conducive to the expression of the binding

molecules or functional variants, and b) optionally, recovering the expressed binding molecules or functional variants. The expressed binding molecules or functional variants thereof can be recovered from the cell free extract, but preferably they are recovered from the culture medium. Methods to recover proteins, such as binding molecules, from cell free extracts or culture medium are well known to the man skilled in the art. Binding molecules or functional variants thereof as obtainable by the above described method are also a part of the present invention. Alternatively, next to the expression in hosts, such as host cells, the binding molecules or functional variants thereof can be produced synthetically by conventional peptide synthesizers or in cell-free translation systems using RNA nucleic acid derived from DNA molecules according to the invention. Binding molecule or functional variants thereof as obtainable by the above described synthetic production methods or cell-free translation systems are also a part of the present invention. In yet another alternative embodiment, binding molecules according to the present invention, preferably human binding molecules specifically binding to a coronavirus, such as SARS-CoV, or a fragment thereof, may be generated by transgenic non-human mammals, such as for instance transgenic mice or rabbits, that express human immunoglobulin genes. Preferably, the transgenic non-human mammals have a genome comprising a human heavy chain transgene and a human light chain transgene encoding all or a portion of the human binding molecules as described above. The transgenic non-human mammals can be immunized with a purified or enriched preparation of a coronavirus, such as SARS-CoV, or a fragment thereof. Protocols for immunizing non-human mammals are well established in the art. See Using Antibodies: A Laboratory

Manual, Edited by: E. Harlow, D. Lane (1998), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York and Current Protocols in Immunology, Edited by: J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober (2001),
5 John Wiley & Sons Inc., New York. Immunization protocols often include multiple immunizations, either with or without adjuvants such as Freund's complete adjuvant and Freund's incomplete adjuvant, but may also include naked DNA
10 immunizations. In another embodiment, the human binding molecules are produced by B cells or plasma cells derived from the transgenic animals or human subjects that have been exposed to SARS-CoV. In yet another embodiment, the human binding molecules are produced by hybridomas which are prepared by fusion of B cells obtained from the above
15 described transgenic non-human mammals or human subjects to immortalized cells. B cells, plasma cells and hybridomas as obtainable from the above described transgenic non-human mammals or human subjects and human binding molecules as obtainable from the above described transgenic non-human
20 mammals or human subjects are also a part of the present invention.

Methods of identifying binding molecules, preferably human binding molecules such as human monoclonal antibodies or fragments thereof, or nucleic acid molecules encoding the
25 binding molecules may comprise the steps of a) contacting a phage library of binding molecules, preferably human binding molecules, with a coronavirus, such as SARS-CoV, or a fragment thereof, b) selecting at least once for a phage binding to the coronavirus or the fragment thereof, and c) separating and
30 recovering the phage binding to the coronavirus or the fragment thereof. The selection step may be performed by

contacting a phage library with a coronavirus which is inactivated. The coronavirus may be isolated or non-isolated, e.g. present in serum and/or blood of an infected individual. Alternatively, the selection step may be performed in the presence of a fragment of a coronavirus such as an extracellular part of the coronavirus (such as SARS-CoV), one or more proteins or (poly)peptides derived from a coronavirus, fusion proteins comprising these proteins or (poly)peptides, and the like. Phage display methods for identifying and obtaining binding molecules, e.g. antibodies, are by now well-established methods known by the person skilled in the art. They are e.g. described in US Patent Number 5,696,108; Burton and Barbas, 1994; de Kruif et al., 1995b; and Phage Display: A Laboratory Manual. Edited by: CF Barbas, DR Burton, JK Scott and GJ Silverman (2001), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. For the construction of phage display libraries, collections of human monoclonal antibody heavy and light chain variable region genes are expressed on the surface of bacteriophage, preferably filamentous bacteriophage, particles, in for example single-chain Fv (scFv) or in Fab format (see de Kruif et al., 1995b). Large libraries of antibody fragment-expressing phages typically contain more than 1.0×10^9 antibody specificities and may be assembled from the immunoglobulin V regions expressed in the B lymphocytes of non-immunized or immunized individuals. In a specific embodiment the phage library of binding molecules, preferably scFv phage library, is prepared from RNA isolated from cells obtained from a subject that has been vaccinated or exposed to a coronavirus, such as SARS-CoV. RNA can be isolated from *inter alia* bone marrow or peripheral blood, preferably peripheral blood

lymphocytes. The subject can be an animal vaccinated or exposed to a coronavirus, but is preferably a human subject which has been vaccinated or has been exposed to a coronavirus. Preferably, the human subject has recovered from
5 the coronavirus.

Alternatively, phage display libraries may be constructed from immunoglobulin variable regions that have been partially assembled *in vitro* to introduce additional antibody diversity in the library (semi-synthetic libraries). For example, *in*
10 *vitro* assembled variable regions contain stretches of synthetically produced, randomized or partially randomized DNA in those regions of the molecules that are important for antibody specificity, e.g. CDR regions. Coronavirus specific phage antibodies can be selected from libraries by
15 immobilising a coronavirus (in inactivated or active form) or target antigens such as antigens from a coronavirus on a solid phase and subsequently exposing the coronavirus (in inactivated or active form) or target antigens to a phage library to allow binding of phages expressing antibody
20 fragments specific for the solid phase-bound antigen(s). Non-bound phages are removed by washing and bound phages eluted from the solid phase for infection of *Escherichia coli* (*E.coli*) bacteria and subsequent propagation. Multiple rounds of selection and propagation are usually required to
25 sufficiently enrich for phages binding specifically to the coronavirus (in inactivated or active form) or target antigen(s). If desired, before exposing the phage library to a coronavirus (in inactivated or active form) or target antigens the phage library can first be subtracted by exposing the
30 phage library to non-target antigens bound to a solid phase. Phages may also be selected for binding to complex antigens such as complex mixtures of coronavirus proteins or

(poly)peptides or host cells expressing one or more proteins or (poly)peptides of a coronavirus. Antigen specific phage antibodies can be selected from the library by incubating a solid phase with bound thereon a preparation of an inactivated coronavirus with the phage antibody library to let for example the scFv or Fab part of the phage bind to the proteins/polypeptides of the coronavirus preparation. After incubation and several washes to remove unbound and loosely attached phages, the phages that have bound with their scFv or Fab part to the preparation are eluted and used to infect *Escherichia coli* to allow amplification of the new specificity. Generally, one or more selection rounds are required to separate the phages of interest from the large excess of non-binding phages. Alternatively, known proteins or (poly)peptides of the coronavirus can be expressed in host cells and these cells can be used for selection of phage antibodies specific for the proteins or (poly)peptides. A phage display method using these host cells can be extended and improved by subtracting non-relevant binders during screening by addition of an excess of host cells comprising no target molecules or non-target molecules that are similar, but not identical, to the target, and thereby strongly enhance the chance of finding relevant binding molecules (This process is referred to as the MabstratTM process. MabstratTM is a pending trademark application of Crucell Holland B.V., see also US Patent Number 6,265,150). An example of a coronavirus against which binding molecules can be found using the above described method of identification is SARS-CoV.

A method of obtaining a binding molecule, preferably a human binding molecule or a nucleic acid molecule encoding such a binding molecule may comprise the steps of a)

performing the above described method of identifying binding molecules, preferably human binding molecules such as human monoclonal antibodies or fragments thereof, or nucleic acid molecules encoding the binding molecules, and b) isolating
5 from the recovered phage the human binding molecule and/or the nucleic acid encoding the human binding molecule. Once a new monoclonal phage antibody has been established or identified with the above mentioned method of identifying binding molecules or nucleic acid molecules encoding the binding
10 molecules, the DNA encoding the scFv or Fab can be isolated from the bacteria or phages and combined with standard molecular biological techniques to make constructs encoding bivalent scFvs or complete human immunoglobulins of a desired specificity (e.g. IgG, IgA or IgM). These constructs can be
15 transfected into suitable cell lines and complete human monoclonal antibodies can be produced (see Huls *et al.*, 1999; Boel *et al.*, 2000).

In addition to the at least two binding molecule, the compositions of the invention may comprise *inter alia*
20 stabilising molecules, such as albumin or polyethylene glycol, or salts. Preferably, the salts used are salts that retain the desired biological activity of the binding molecules and do not impart any undesired toxicological effects. Examples of such salts include, but are not limited to, acid addition
25 salts and base addition salts. Acid addition salts include, but are not limited to, those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and
30 dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include, but

are not limited to, those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like. If necessary, the binding molecules of the invention may be coated in or on a material to protect them from the action of acids or other natural or non-natural conditions that may inactivate the binding molecules.

In yet a further aspect, the invention provides compositions comprising at least two nucleic acid molecule encoding binding molecules as defined in the present invention. The compositions may comprise aqueous solutions such as aqueous solutions containing salts (e.g., NaCl or salts as described above), detergents (e.g., SDS) and/or other suitable components.

Furthermore, the present invention pertains to pharmaceutical compositions comprising a composition according to the invention. The pharmaceutical composition of the invention further comprises at least one pharmaceutically acceptable excipient.

A pharmaceutical composition according to the invention can further comprise at least one other therapeutic, prophylactic and/or diagnostic agent. Preferably, said further therapeutic and/or prophylactic agents are agents capable of preventing and/or treating an infection and/or a condition resulting from a coronavirus, such as SARS-CoV. Therapeutic and/or prophylactic agents include, but are not limited to, anti-viral agents. Such agents can be binding molecules, small molecules, organic or inorganic compounds, enzymes, polynucleotide sequences etc.

Examples of anti-viral agents are well known to the skilled artisan. Agents that are currently used to treat patients infected with for instance SARS-CoV are interferon-alpha, steroids and potential replicase inhibitors.

5 Furthermore, patients infected with SARS-CoV are currently treated by transfusion of serum produced from blood donated by recovering/recovered SARS patients who have produced antibodies after being exposed to the virus. Agents capable of preventing and/or treating an infection with SARS-CoV or other
10 coronavirus and/or a condition resulting from SARS-CoV or other coronavirus that are in the experimental phase might also be used as other therapeutic and/or prophylactic agents useful in the present invention.

The pharmaceutical compositions of the invention can be
15 tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, ferrets, mice, rats, chicken, cows, monkeys, pigs, dogs, rabbits, etc.

Typically, pharmaceutical compositions must be sterile
20 and stable under the conditions of manufacture and storage. The compositions of the present invention can be in powder form for reconstitution in the appropriate pharmaceutically acceptable excipient before or at the time of delivery. In the case of sterile powders for the preparation of sterile
25 injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

30 Alternatively, the compositions of the present invention can be in solution and the appropriate pharmaceutically acceptable excipient can be added and/or mixed before or at

the time of delivery to provide a unit dosage injectable form. Preferably, the pharmaceutically acceptable excipient used in the present invention is suitable to high drug concentration, can maintain proper fluidity and, if necessary, can delay
5 absorption.

The choice of the optimal route of administration of the pharmaceutical compositions will be influenced by several factors including the physico-chemical properties of the active molecules within the compositions, the urgency of the
10 clinical situation and the relationship of the plasma concentrations of the active molecules to the desired therapeutic effect. For instance, if necessary, the compositions of the invention can be prepared with carriers that will protect them against rapid release, such as a
15 controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can *inter alia* be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.
20 Furthermore, it may be necessary to coat the compositions with, or co-administer the compositions with, a material or compound that prevents the inactivation of the binding molecules in the compositions. For example, the binding molecules of the compositions may be administered to a subject
25 in an appropriate carrier, for example, liposomes, or a diluent.

The routes of administration can be divided into two main categories, oral and parenteral administration. These two categories include several routes of administration well known
30 to the skilled person. The preferred administration route is intravenous, particularly preferred is intramuscular.

Oral dosage forms can be formulated in several formulations and may contain pharmaceutically acceptable excipients including, but not limited to, inert diluents, granulating and disintegrating agents, binding agents, lubricating agents, preservatives, colouring, flavouring or sweetening agents, vegetable oils, mineral oils, wetting agents, and thickening agents.

The pharmaceutical compositions of the present invention can also be formulated for parenteral administration.

Formulations for parenteral administration can be *inter alia* in the form of aqueous or non-aqueous isotonic sterile non-toxic injection or infusion solutions or suspensions. The solutions or suspensions may comprise agents that are non-toxic to recipients at the dosages and concentrations employed. Such agents are well known to the skilled artisan and include 1,3-butanediol, Ringer's solution, Hank's solution, isotonic sodium chloride solution, oils or fatty acids, local anaesthetic agents, preservatives, buffers, viscosity or solubility increasing agents, water-soluble antioxidants, oil-soluble antioxidants, and metal chelating agents.

In a further aspect, the pharmaceutical compositions of the invention can be used as a medicament. So, a method of treatment and/or prevention of a coronavirus infection using the pharmaceutical compositions of the invention is another part of the present invention. The (pharmaceutical) compositions of the invention can *inter alia* be used in the diagnosis, prophylaxis, treatment, or combination thereof, of one or more conditions resulting from a coronavirus. They are suitable for treatment of yet untreated patients suffering from a condition resulting from a coronavirus and patients who have been or are treated from a condition resulting from a

coronavirus. They protect against further infection by a coronavirus and/or will retard the onset or progress of the symptoms associated with a coronavirus. They may even be used in the prophylaxis of conditions resulting from a coronavirus in for instance people exposed to the coronavirus such as hospital personnel taking care of suspected patients. Preferably, the (pharmaceutical) compositions can be used in a method to detect, prevent, and/or treat a human coronavirus, such as SARS-CoV, infection.

The above mentioned compositions and pharmaceutical compositions may be employed in conjunction with other molecules useful in diagnosis, prophylaxis and/or treatment of a coronavirus infection. They can be used *in vitro*, *ex vivo* or *in vivo*. For instance, the pharmaceutical compositions of the invention can be co-administered with a vaccine against a coronavirus, such as SARS-CoV. Alternatively, the vaccine may also be administered before or after administration of the pharmaceutical compositions of the invention. Administration of the pharmaceutical compositions of the invention with a vaccine might be suitable for postexposure prophylaxis and might also decrease possible side effects of a live-attenuated vaccine in immunocompromised recipients.

The binding molecules are typically formulated in the compositions and pharmaceutical compositions of the invention in a therapeutically or diagnostically effective amount. Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). A suitable dosage range may for instance be 0.05-100 mg/kg body weight, preferably 0.1-15 mg/kg body weight.

Typically, the molar ratio of the two binding molecules in the compositions and pharmaceutical compositions of the invention may vary from 1:100 to 100:1, preferably from 1:50

to 50:1, more preferably from 1:25 to 25:1, particularly 1:10 to 10:1, and more particularly 1:5 to 5:1. Furthermore, for example, a single bolus may be administered, several divided doses may be administered over time or the dose may be

5 proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The molecules and compositions according to the present invention are preferably sterile. Methods to render these molecules and compositions sterile are well known in the art. The other molecules useful

10 in diagnosis, prophylaxis and/or treatment can be administered in a similar dosage regimen as proposed for the binding molecules of the invention. If the other molecules are administered separately, they may be administered to a patient prior to (e.g., 2 minutes, 5 minutes, 10 minutes, 15 minutes,

15 30 minutes, 45 minutes, 60 minutes, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 2 weeks, 4 weeks or 6 weeks before), concomitantly with, or subsequent to (e.g., 2 minutes, 5 minutes, 10 minutes, 15

20 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 2 weeks, 4 weeks or 6 weeks after) the administration of one or more of the binding molecules or

25 pharmaceutical compositions of the invention. The exact dosing regimen is usually sorted out during clinical trials in human patients.

Human binding molecules and pharmaceutical compositions comprising the human binding molecules are particularly

30 useful, and often preferred, when to be administered to human beings as *in vivo* therapeutic agents, since recipient immune response to the administered antibody will often be

substantially less than that occasioned by administration of a monoclonal murine, chimeric or humanised binding molecule.

In another aspect, the invention concerns the use of (pharmaceutical) compositions according to the invention in
5 the preparation of a medicament for the diagnosis, prophylaxis, treatment, or combination thereof, of a condition resulting from a coronavirus. Preferably, the coronavirus is a human coronavirus such as SARS-CoV.

Next to that, kits comprising at least one composition
10 according to the invention or at least one pharmaceutical composition according to the invention are also a part of the present invention. Optionally, the above described components of the kits of the invention are packed in suitable containers and labeled for diagnosis, prophylaxis and/or treatment of the
15 indicated conditions. The binding molecules in the (pharmaceutical) compositions may be packaged individually. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile,
20 solution or as a lyophilized, preferably sterile, formulation for reconstitution. The containers may be formed from a variety of materials and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection
25 needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of
30 the suitable hosts. Associated with the kits can be instructions customarily included in commercial packages of therapeutic, prophylactic or diagnostic products, that contain

information about for example the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic, prophylactic or diagnostic products.

5 The invention further pertains to a method of detecting a SARS-CoV in a sample, wherein the method comprises the steps of a) contacting a sample with a diagnostically effective amount of compositions or pharmaceutical compositions according to the invention, and b) determining whether the
10 compositions or pharmaceutical compositions specifically bind to a molecule of the sample. The sample may be a biological sample including, but not limited to blood, serum, urine, tissue or other biological material from (potentially) infected subjects, or a nonbiological sample such as water,
15 drink, etc. The (potentially) infected subjects may be human subjects, but also animals that are suspected as carriers of a coronavirus, such as SARS-CoV, might be tested for the presence of the coronavirus using the compositions or pharmaceutical compositions. The sample may first be
20 manipulated to make it more suitable for the method of detection. Manipulation mean *inter alia* treating the sample suspected to contain and/or containing the coronavirus in such a way that the coronavirus will disintegrate into antigenic components such as proteins, (poly)peptides or other antigenic
25 fragments. Preferably, the compositions or pharmaceutical compositions are contacted with the sample under conditions which allow the formation of an immunological complex between the binding molecules in the compositions or pharmaceutical compositions and the coronavirus or antigenic components
30 thereof that may be present in the sample. The formation of an immunological complex, if any, indicating the presence of the coronavirus in the sample, is then detected and measured by

suitable means. Such methods include, *inter alia*, homogeneous and heterogeneous binding immunoassays, such as radioimmunoassays (RIA), ELISA, immunofluorescence, immunohistochemistry, FACS, BIACORE and Western blot analyses.

5 Preferred assay techniques, especially for large-scale clinical screening of patient sera and blood and blood-derived products are ELISA and Western blot techniques. ELISA tests are particularly preferred and well known to persons skilled in the art.

10 In a further aspect, the invention provides a method of screening a binding molecule or a functional variant of a binding molecule for specific binding to a different, non-overlapping epitope of a coronavirus such as SARS-CoV as the epitope bound by a binding molecule or functional variant of
15 the invention, wherein the method comprises the steps of a) contacting a binding molecule or a functional variant to be screened, a binding molecule or functional variant of the invention and a coronavirus or fragment thereof, b) measure if the binding molecule or functional variant to be screened is
20 capable of competing for specifically binding to the coronavirus or fragment thereof with the binding molecule or functional variant of the invention. If the binding molecule or functional variant to be screened is not capable of competing for specifically binding to the coronavirus or
25 fragment thereof with the binding molecule or functional variant of the invention, it most likely binds to a different, non-overlapping epitope. In a further step it may be determined if the screened binding molecules that bind to a different, non-overlapping epitope compared to the binding
30 molecules of the invention have coronavirus neutralizing activity. In yet a further step it can be determined if the screened binding molecules that bind to a different, non-

overlapping epitope compared to the binding molecules of the invention and have coronavirus neutralizing activity form together with the binding molecules of the invention a composition exhibiting synergistic coronavirus neutralizing activity. Assays to screen for non-competing binding molecules and measure (synergistic) neutralizing activity are well known to the skilled person.

EXAMPLES

10 To illustrate the invention, the following examples are provided. The examples are not intended to limit the scope of the invention in any way.

Example 1

15 *Construction of a scFv phage display library using peripheral blood lymphocytes of a patient having been exposed to SARS-CoV*

Lymphocytes were obtained from a patient recovered from SARS-CoV (see Rickerts *et al.* 2003) and frozen in liquid nitrogen. The lymphocytes were quickly thawed in a 37°C water bath and transferred to wet-ice. The lymphocytes were diluted with cold DMEM culture medium to a final volume of 50 ml in a 50 ml tube and centrifuged for 5 minutes at 350xg. The obtained cell pellet was suspended in 5 ml DMEM and cell density was determined microscopically using trypan-blue exclusion to visualize dead cells. All cells ($\sim 5 \times 10^6$) were spun again for 5 minutes at 350xg, decanted and suspended in residual fluid (DMEM). Total RNA was prepared from these cells using organic phase separation (TRIZOLTM) and subsequent ethanol precipitation. The obtained RNA was dissolved in DEPC treated ultrapure water and the concentration was determined by OD 260 nm measurement. Thereafter, the RNA was diluted to a concentration of 100 ng/ μ l. Next, 1 μ g of RNA was converted

into cDNA as follows: To 10 μ l total RNA, 13 μ l DEPC treated ultrapure water and 1 μ l random hexamers (500 ng/ μ l) were added and the obtained mixture was heated at 65°C for 5 minutes and quickly cooled on wet-ice. Then, 8 μ l 5X First-
5 Strand buffer, 2 μ l dNTP (10 mM each), 2 μ l DTT (0.1 M), 2 μ l Rnase-inhibitor (40 U/ μ l) and 2 μ l SuperscriptTMIII MMLV reverse transcriptase (200 U/ μ l) were added to the mixture, incubated at room temperature for 5 minutes and incubated for 1 hour at 50°C. The reaction was terminated by heat
10 inactivation, *i.e.* by incubating the mixture for 15 minutes at 75°C.

The obtained cDNA products were diluted to a final volume of 200 μ l with DEPC treated ultrapure water. The OD 260 nm of a 50 times diluted solution (in 10 mM Tris buffer) of the
15 dilution of the obtained cDNA products gave a value of 0.1.

5 to 10 μ l of the diluted cDNA products were used as template for PCR amplification of the immunoglobulin gamma heavy chain family and kappa or lambda light chain sequences using specific oligonucleotide primers (see Tables 2-9). PCR
20 reaction mixtures contained, besides the diluted cDNA products, 25 pmol sense primer and 25 pmol anti-sense primer in a final volume of 50 μ l of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 250 μ M dNTPs and 1.25 units Taq polymerase. In a heated-lid thermal cycler having a temperature of 96°C,
25 the mixtures obtained were quickly melted for 2 minutes, followed by 30 amplification cycles of: 30 seconds at 96°C, 30 seconds at 60°C and 60 seconds at 72°C. In a first round amplification, each of nine sense directed primers (see Table 2; covering all families of heavy chain variable regions) was
30 combined with an IgG specific constant region anti-sense primer called HuCIgG 5'-GTC CAC CTT GGT GTT GCT GGG CTT-3' (SEQ ID NO:87) yielding nine products of about 650 basepairs.

These products were purified on a 2% agarose gel and isolated from the gel using Qiagen gel-extraction columns. 1/10 of each of the isolated products was used in an identical PCR reaction as described above using the same nine sense primers (covering all families of heavy chain variable regions), whereby each sense primer was combined with one of the four J-region specific anti-sense primers (see Table 3). This resulted in 36 products of approximately 350 basepairs. The products obtained were purified on a 2% agarose gel and isolated from the gel using Qiagen gel-extraction columns. In a third round, 1/10 of each of the isolated products was subjected to re-amplification with the same set of primers as in the second round with the proviso that the primers used were extended with restriction sites (see Table 4) to enable directed cloning in the phage display vector pDV-C05 (see SEQ ID NO:88). This resulted again in 36 products. These products were pooled per used (VH) sense primer into nine fractions. In the next step, 2.5 µg of pooled fraction and 100 µg pDV-C05 vector were digested with NcoI and XhoI and purified by gel. Thereafter, a ligation was performed overnight at 16°C as follows. To 500 ng pDV-C05 vector 70 ng pooled fraction was added in a total volume of 50 µl ligation mix containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, 25 µg/ml BSA and 2.5 µl T4 DNA Ligase (400 u/µl). This procedure was followed for each pooled fraction. The ligation mixes were purified by phenol/chloroform, followed by a chloroform extraction and ethanol precipitation, methods well known to the skilled artisan. The DNA obtained was dissolved in 50 µl ultrapure water and per ligation mix two times 2.5 µl aliquots were electroporated into 40 µl of TG1 competent *E. coli* bacteria according to the manufacturer's protocol (Stratagene). Transformants were grown overnight at 37°C in a

total of 27 dishes (three dishes per pooled fraction;
dimension of dish: 240 mm x 240 mm) containing 2TY agar
supplemented with 50 µg/ml ampicillin and 4.5% glucose. A
(sub)library of variable heavy chain regions was obtained by
5 scraping the transformants from the agar plates. This
(sub)library was directly used for plasmid DNA preparation
using a QiagenTM kit.

The light chain immunoglobulin sequences were amplified
from the same cDNA preparation in a similar three round PCR
10 procedure and identical reaction parameters as described above
for the heavy chain regions with the proviso that the primers
depicted in Tables 5-9 were used. The first amplification was
performed using a set of seventeen light chain variable region
sense primers (eleven for the lambda light chain (see Table 5)
15 and six for the kappa light chain (see Table 6)) each combined
with an anti-sense primer recognizing the C-kappa called HuCk
5'-ACACTCTCCCCTGTTGAAGCTCTT-3' (see SEQ ID NO:89) or C-lambda
constant region HuCλ2 5'-TGAACATTCTGTAGGGGCCACTG-3' (see SEQ
ID NO:90) or HuCλ7 5'-AGAGCATTCTGCAGGGGCCACTG-3' (see SEQ ID
20 NO:91) (the HuCλ2 and HuCλ7 anti-sense primers were mixed to
equimolarity before use), yielding 17 products of about 600
basepairs. These products were purified on a 2% agarose gel
and isolated from the gel using Qiagen gel-extraction columns.
1/10 of each of the isolated products was used in an identical
25 PCR reaction as described above using the same seventeen sense
primers, whereby each lambda light chain sense primer was
combined with one of the three Jlambda-region specific anti-
sense primers (see Table 7) and each kappa light chain sense
primer was combined with one of the five Jkappa-region
30 specific anti-sense primers (see Table 8). This resulted in 63
products of approximately 350 basepairs. The products obtained
were purified on a 2% agarose gel and isolated from the gel

using Qiagen gel-extraction columns. In a third round, 1/10 of each of the isolated products was subjected to re-amplification with the same set of primers as in the second round with the proviso that the primers used were extended with restriction sites (see Table 9) to enable directed cloning in the heavy chain (sub)library vector. This resulted again in 63 products. These products were pooled to a total of 10 fractions. This number of fractions was chosen to maintain the natural distribution of the different light chain families within the library and to over or under represent certain families. The number of alleles within a family was used to determine the percentage of representation within a library (see Table 10). Next, the fractions were digested with SalI and NotI and ligated in the heavy chain (sub)library vector, which was cut with the same restriction enzymes, using the same ligation procedure and volumes as described above for the heavy chain (sub)library. Ligation purification and subsequent transformation of the resulting definitive library was also performed as described above for the heavy chain (sub)library. The transformants were grown in 30 dishes (three dishes per pooled fraction; dimension of dish: 240 mm x 240 mm) containing 2TY agar supplemented with 50 µg/ml ampicillin and 4.5% glucose. All bacteria were harvested in 2TY culture medium containing 50 µg/ml ampicillin and 4.5% glucose, mixed with glycerol to 15% (v/v) and frozen in 1.5 ml aliquots at -80°C.

Example 2

Selection of phage carrying single-chain Fv fragments specifically recognizing SARS-CoV

Antibody fragments were selected using antibody phage display libraries and technology, essentially as described in

US patent 6,265,150 and in WO 98/15833. All procedures were performed at room temperature unless stated otherwise. An inactivated SARS-CoV preparation (Frankfurt 1 strain) was prepared as follows. Medium from Vero cells which were
5 infected with SARS-CoV strain Frankfurt 1 was harvested as soon as cytopathic effect (CPE) was observed. Cell debris was removed by centrifugation of the harvested medium for 15 minutes at 3000 rpm. The obtained supernatant was collected, spun again for 15 minutes at 3000 rpm and transferred to a
10 clean tube. Subsequently, ultracentrifuge tubes were filled with 10 ml sterile 25% glycerol in PBS. 20 ml of the cleared supernatant was gently applied on the glycerol cushion and the tubes were spun for 2 hours at 20,000 rpm at 4°C. The supernatant was discarded and the virus pellets were
15 resuspended in 1 ml TNE buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 200 mM NaCl) and stored at -80°C. Next, the resuspended virus pellets were gamma-irradiated at 45kGy on dry ice. Subsequently, they were tested for the absence of infectivity in cell culture. If absence of infectivity was established,
20 the thus obtained inactivated SARS-CoV preparation was used for selection of single-chain phage antibodies specifically binding to SARS-CoV.

The inactivated virus preparation and heat-inactivated fetal bovine serum (FBS) were coated overnight at 4°C onto the
25 surface of separate MaxisorpTM plastic tubes (Nunc). The tubes were blocked for two hours in 3 ml PBS containing 2% FBS and 2% fat free milk powder (2% PBS-FM). After two hours the FBS-coated tube was emptied and washed three times with PBS. To this tube, 500 µl (approximately 10¹³ cfu) of a phage display
30 library expressing single-chain Fv fragments (scFvs) essentially prepared as described by De Kruif et al. (1995a)

and references therein, 500 μ l 4% PBS-FM and 2 ml 2% PBS-FM were added. The tube was sealed and rotated slowly at room temperature for two hours. Subsequently, the obtained blocked phage library (3 ml) was transferred to a SARS-CoV
5 preparation-coated tube that had been washed three times with PBS. Tween*-20 was added to a final concentration of 0.05% and binding was allowed to proceed for two hours on a slowly rotating wheel at room temperature or at 37°C. The tube was emptied and washed ten times with PBS containing 0.05% Tween-
10 20, followed by washing ten times with PBS. 1 ml glycine-HCL (0.05 M, pH 2.2) was added to elute bound phages, and the tube was rotated slowly for ten minutes. For neutralisation purposes, the eluted phages were added to 500 μ l 1 M Tris-HCL pH 7.4. To this mixture, 5 ml of exponentially growing XL-1
15 blue bacterial culture was added. The obtained culture was incubated for thirty minutes at 37°C without shaking. Then, the bacteria were plated on TYE agar plates containing ampicillin, tetracycline and glucose. After overnight incubation of the plates at 37°C, the colonies were scraped
20 from the plates and used to prepare an enriched phage library, essentially as described by De Kruif et al. (1995a) and WO 02/103012. Briefly, scraped bacteria were used to inoculate 2TY medium containing ampicillin, tetracycline and glucose and grown at a temperature of 37°C to an OD 600 nm of ~0.3. CT or
25 VCSM13 helper phages were added and allowed to infect the bacteria after which the medium was changed to 2TY containing ampicillin, tetracycline and kanamycin. Incubation was continued overnight at 30°C. The next day, the bacteria were removed from the 2TY medium by centrifugation after which the
30 phages in the obtained supernatant were precipitated using

*Trade-mark

polyethylene glycol 6000/NaCl. Finally, the phages were dissolved in a small volume of PBS containing 1% BSA, filter-sterilized and used for a next round of selection. The selection/re-infection procedure was performed two or three
5 times. After each round of selection, individual *E.coli* colonies were used to prepare monoclonal phage antibodies. Essentially, individual colonies were grown to log-phase and infected with VCSM13 helper phages after which phage antibody production was allowed to proceed overnight. Phage antibody
10 containing supernatants were tested in ELISA for binding activity to the SARS-CoV preparation which was coated to 96-well plates. In the above-described selection, the phage antibody called SC03-014 was obtained. ScFvs of the phage antibody SC03-014 were produced as described before in De
15 Kruif *et al.* (1995a and 1995b) and references therein. The buffer of the scFvs was adjusted to 1 x PBS.

Additionally, antibody fragments were selected from the immune phage display library expressing single chain Fv fragments (scFvs) (see Example 1 for the construction of this
20 library) essentially as described *supra*. For the selection described below an UV-inactivated SARS-CoV preparation was used. UV-inactivated SARS-CoV (Frankfurt 1 strain) was prepared as follows. Medium from Vero cells which were infected with O.I. moi (multiplicity of infection) SARS-CoV
25 strain Frankfurt 1 was harvested as soon as cytopathic effect (CPE) was observed. Cells were once frozen at -20°C and thawed. Cell debris was removed by centrifugation of the harvested medium for 15 minutes at 3000 rpm. The obtained supernatant was collected, spun again for 15 minutes at 3000
30 rpm and transferred to a clean tube. Subsequently, ultracentrifuge tubes were filled with 10 ml sterile 25% v/v

glycerol in PBS. 20 ml of the cleared supernatant was gently applied on the glycerol cushion and the tubes were spun for 2 hours at 20,000 rpm at 4°C in a Beckman SW28 rotor. The supernatant was discarded and the virus pellets were
5 resuspended in 1 ml TNE buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 200 mM NaCl) and stored at -80°C. Next, the resuspended virus pellets were UV-irradiated at 4°C for 15 minutes (UV-B radiation 280-350 nm; λ_{max} 306 nm). Subsequently, they were tested for the absence of infectivity in cell culture. If
10 absence of infectivity was established, the thus obtained inactivated SARS-CoV preparations were used for further experiments.

In contrast to the selections described *supra* no pre-subtraction using heat-inactivated fetal bovine serum coated
15 MaxisorpTM tubes (Nunc) was performed. To the SARS-CoV coated tubes, 500 μ l (approximately 10^{13} cfu) of the immune phage display library expressing single chain Fv fragments (scFvs), one volume of 4% PBS-FM and Tween-20 to a final concentration of 0.05% was added.

20 For the immune phage display library selections which consisted of a single selection round only, binding was allowed to proceed at 37°C or room temperature on a slowly rotating wheel at 37°C followed by an incubation of 30 minutes without agitation. The following selections and washes were
25 performed: incubation at 37°C, washing 5 times with PBS containing 0.05% Tween-20 (PBST) and 5 times with PBS; incubation at 37°C, washing 10 times with PBST and 10 times with PBS; incubation at room temperature, washing 10 times with PBST and 10 times with PBS. Bound phages were eluted and
30 processed as described above. Phages derived from individual colonies were tested in ELISA for binding activity to SARS-CoV coated to 96-well plates. In the selections from the immune

phage display library the phage antibody called SC03-022 was obtained.

Example 3

5 *Validation of the SARS-CoV specific single-chain phage antibodies*

Selected single-chain phage antibodies that were obtained in the screens described above, were validated in ELISA for specificity, *i.e.* binding to the UV-inactivated SARS-CoV preparation prepared as described *supra*. Additionally, the single-chain phage antibodies were also tested for binding to 10% FBS. For this purpose, the UV-inactivated SARS-CoV preparation or 10% FBS preparation was coated to Maxisorp™ ELISA plates. After coating, the plates were blocked in 2% PBS-FM. The selected single-chain phage antibodies were incubated in an equal volume of 4% PBS-FM to obtain blocked phage antibodies. The plates were emptied, washed three times with PBS, after which the blocked phage antibodies were added. Incubation was allowed to proceed for one hour, the plates were washed in PBS containing 0.05% Tween-20 and bound phage antibodies were detected (using OD 492 nm measurement) using an anti-M13 antibody conjugated to peroxidase. As a control, the procedure was performed simultaneously using no single-chain phage antibody or control single-chain phage antibody directed against thyroglobulin (SC02-006) (see De Kruif *et al.* 1995a and 1995b). Both controls served as a negative control. As shown in Figure 1, the selected phage antibodies called SC03-014 and SC03-022 displayed significant binding to the immobilized UV-inactivated SARS-CoV preparation, while no binding to FBS was observed.

Example 4

Characterization of the scFvs specific for SARS-CoV.

From the selected specific single chain phage antibody (scFv) clones plasmid DNA was obtained and nucleotide sequences were determined according to standard techniques.

5 The nucleotide sequences of the scFvs (including restriction sites for cloning) called SC03-014 and SC03-022 are shown in SEQ ID NO:92 and SEQ ID NO:94, respectively. The amino acid sequences of the scFvs called SC03-014 and SC03-022 are shown in SEQ ID NO:93 and SEQ ID NO:95, respectively. The VH and VL
 10 gene identity (see Tomlinson IM, Williams SC, Ignatovitch O, Corbett SJ, Winter G. V-BASE Sequence Directory. Cambridge United Kingdom: MRC Centre for Protein Engineering (1997)) and heavy chain CDR3 compositions of the scFvs specifically binding the SARS-CoV preparation are depicted in Table 11.

15

Example 5

Construction of fully human immunoglobulin molecules (human monoclonal anti-SARS-CoV antibodies) from the selected anti-SARS-CoV single chain Fvs

20 Heavy and light chain variable regions of the scFvs called SC03-014 and SC03-022 were PCR-amplified using oligonucleotides to append restriction sites and/or sequences for expression in the IgG expression vectors pSyn-C03-HC γ 1 (see SEQ ID No:96) and pSyn-C05-Ck (see SEQ ID No:97),
 25 respectively. The V_L gene of scFv SC03-014 was amplified using oligonucleotides 5K-I acctgtctcgagttttccatggctgacatccagat gaccagttctccatcctcc (SEQ ID NO:98) and sy3K-C gctggggggcggccac ggtccgtttgatctccaccttggtccc (SEQ ID NO:99) and the PCR product cloned into vector pSyn-C05-Ck. The V_L gene of scFv SC03-022
 30 was amplified using oligonucleotides 5K-J acctgtctcgagt tttccatggctgacatcgtgatgaccagttctccag (SEQ ID NO:100) and sy3K-F gctggggggcggccacggtccgcttgatctccaccttggtccc (SEQ ID NO:101)

and the PCR product cloned into vector pSyn-C05-Ck. Nucleotide sequences for all constructs were verified according to standard techniques known to the skilled artisan. V_H genes of scFv SC03-014 were amplified using oligonucleotides 5H-B
 5 acctgtcttgaattctccatggccgaggtgcagctggtggagtctg (SEQ ID NO:102) and sy3H-A gcccttggtgctagcgctggagacgggtcaccaggggtgccctggcccc (SEQ ID NO:103). V_H genes of scFv SC03-022 were amplified using oligonucleotide set 5H-H acctgtcttgaattctccatggccgaggtgcag
 ctggtgcagtctgg (SEQ ID NO:104) and sy3H-C gcccttggtgctagcgct
 10 ggagacgggtcacgggtggtgccctggcccc (SEQ ID NO:105). Thereafter, the PCR products were cloned into vector pSyn-C03-HCγ1 and nucleotide sequences were verified according to standard techniques known to the skilled person in the art.

The resulting expression constructs pgG103-014C03 and
 15 pgG103-022C03 encoding the anti-SARS-CoV human IgG1 heavy chains were transiently expressed in combination with the pSyn-C05-VkI (V_L SC03-014) and pgG103-022C05 (V_L SC03-022), respectively in HEK293T or PER.C6[®] cells and supernatants containing IgG1 antibodies were obtained. The nucleotide
 20 sequences of the heavy chains of the antibodies called CR03-014 and CR03-022 are shown in SEQ ID NO:106 and SEQ ID NO:108, respectively. The amino acid sequences of the heavy chains of the antibodies CR03-014 and CR03-022 are shown in SEQ ID NO:107 and SEQ ID NO:109, respectively.

25 The nucleotide sequences of the light chain of antibodies CR03-014 and CR03-022 is shown in SEQ ID NO:110 and SEQ ID NO:112, respectively. The amino acid sequences of the light chain of antibodies CR03-014 and CR03-022 is shown in SEQ ID NO:111 and SEQ ID NO:113. Subsequently, the recombinant human
 30 monoclonal antibodies were purified over protein-A columns and size-exclusion columns using standard purification methods

used generally for immunoglobulins (see for instance WO 00/63403).

Example 6

5 *Screening assay for SARS-CoV neutralizing activity of recombinant human anti-SARS-CoV antibodies*

The SARS-CoV neutralization assay was performed on Vero cells (ATCC CCL 81). The SARS-CoV strains used in the neutralization assay were the Frankfurt 1 strain (for the
10 complete genome of this strain see EMBL-database accession # AY291315) (Rickerts et al. 2003). Virus stocks of the strains were used in a titer of 4×10^3 TCID₅₀/ml (50% tissue culture infective dose per ml), with the titer calculated according to the method of Spearman and Kaerber which is known to the
15 average skilled person. Recombinant human anti-SARS-CoV antibodies produced as described above were screened by serially 2-fold-dilution of the undiluted material (2.5 mg/ml) in PBS starting from 1:4 (dilution range 1:4 - 1:512). A neutralization titer of $\geq 1:4$ was regarded as specific
20 evidence of reactivity of the antibodies against SARS-CoV in the screening assay. Convalescent serum from a SARS-patient was used as a positive control for the neutralization assay.

In general, the neutralization assay worked as follows. 25 μ l of the respective antibody dilutions were mixed with 25
25 μ l of virus suspension (= approx. 100 TCID₅₀/25 μ l) and incubated for one hour at 37°C. The suspension was then pipetted in triplicate into 96-well plates. Next, 50 μ l of a freshly trypsinized and homogenized suspension of Vero cells (1:3 split of the confluent cell monolayer of a T75-flask),
30 resuspended in DMEM containing 10% w/v FCS and antibiotics, were added. The inoculated cells were cultured for 3-4 days at 37°C and observed daily for the development of cytopathic

effect (CPE). CPE was compared to the positive control (virus inoculated cells) and negative controls (mock-inoculated cells). The complete absence of CPE in an individual cell culture was defined as protection (= 100% titer reduction).

5 The highest antibody/serum dilution giving protection in 66% percent of wells was defined as the neutralizing antibody titer. The experiment was performed three times in triplicate (see Tables 12A, B and C). The IgGs CR03-014, CR03-022, a negative control IgG1 and a positive control serum from a SARS-patient were tested for SARS-CoV neutralizing activity. It is clear from Table 12A, B and C that the IgGs CR03-014 and CR03-022 displayed significant neutralizing activity. The CR03-014 IgG neutralized the Frankfurt 1 strain at titers of 128 (n=1) or 256 (n=2) in the above described assay. The CR03-022 IgG neutralized the Frankfurt 1 strain at titers of 32 (n=1) or 64 (n=2). These titers correspond to final antibody neutralization concentrations in the tissue culture well of 2.4 µg/ml (n=2) and 4.9 µg/ml (n=1) for CR03-014 and 9.8 µg/ml (n=2) and 19.5 µg/ml (n=1) for CR03-022. In the light of these concentrations both neutralizing antibodies may be suitable in the prophylaxis and/or treatment of a condition resulting from a SARS-CoV infection.

Additionally, different SARS-CoV strains were used to evaluate the potency and breadth of protection of the anti-SARS-CoV antibodies. The SARS-CoV strains HKU-36, HKU-39849, HKU-66, HKU-61567, GZ43 and GZ50 were passaged on FRhK-4 cells for two or three times before testing (see Table 13). Strain HKU-61644 was passaged on Vero cells and tested after passage 1 and 15. The SARS-CoV neutralization assay was performed on FRhK-4 cells as follows. A 500 µl stock solution (100 µg/ml) of antibody was prepared in maintenance medium (MM, MEM supplemented with 1% w/v FCS). From this stock solution 2-

fold-serially dilutions were prepared. 220 μ l stock solution (100 μ g/ml) was added in duplo in a 96-well plate from which 110 μ l was taken and mixed with 110 μ l MM in each of the nine subsequent wells. 110 μ l of the tenth well was discarded. This
5 resulted in ten wells containing 110 μ l 0.2-100 μ g/ml antibody. 110 μ l of the antibody dilution was mixed with 110 μ l of the different SARS-CoV isolates at a concentration of 2000 TCID₅₀/ml with the titer calculated according to the method of Reed and Muench which is known to the skilled
10 artisan. At this stage, in a 220 μ l volume, antibody concentrations varied from 0.1 to 50 μ g/ml in the presence of 1000 TCID₅₀/ml SARS-CoV. The 96-well plate containing the antibody/virus mixtures was preincubated for 1-2 hours at 37°C. 100 μ l of the antibody/virus mixtures were added in
15 quadruplicate to wells from a second 96-well tissue culture plate containing confluent FRhK-4 cells in 100 μ l MM and incubated at 37°C. During this final incubation step, 100 TCID₅₀ of SARS-CoV was present in the presence of antibody concentrations varying from 0.05 to 25 μ g/ml. The cells were
20 cultured at 37°C and observed for the development of CPE at 72 and 96 hours. CPE was compared to a positive control (SARS-CoV inoculated cells) and a negative control (cells incubated with MM only). The antibody neutralization titer was determined as the concentration of antibody which gave 100% protection of
25 the quadruplicate cell cultures. The monoclonal anti-SARS-CoV antibody CR03-014 completely neutralized 100 TCID₅₀ of all tested SARS-CoV isolates at a concentration of 12.5 μ g/ml (see Table 13). This indicates that antibody CR03-014 is able to neutralize a variety of SARS-CoV isolates.

30 In an additional experiment the SARS-CoV neutralization assay was performed as described for the Frankfurt 1 strain *supra* to determine synergy between SARS-CoV neutralizing

antibodies CR03-014 and CR03-022. Stock solutions of antibody CR03-014 and CR03-022 of approximately similar potency were mixed in different ratios. To compensate for an estimated 4 times higher potency of CR03-014 compared to CR03-022, the CR03-014 antibody stock solution of 2.5 mg/ml was diluted 4-fold to 625 µg/ml. Subsequently, antibody CR03-014 and CR03-022 were mixed in the following ratios (mixture A: CR03-014 0%, CR03-022 100%; mixture B: CR03-014 10%, CR03-022 90%; mixture C: CR03-014 50%, CR03-022 50%; mixture D: CR03-014 90%, CR03-022 10%; and mixture E: CR03-014 100%, CR03-022 0%). When the antibodies in the mixtures have an additive effect, the mixtures should neutralize SARS-CoV at the same titer as the individual antibodies present in the mixtures. When the antibodies in the mixtures have a synergistic effect, the mixtures should neutralize SARS-CoV at a higher titer as the individual antibodies present in the mixtures. The neutralization assay was performed twice in triplicate wells as described above. The results of both assays were combined. Protection of at least 66% percent of the wells (4 of the 6 wells tested) was defined as the neutralizing antibody titer. The neutralization titers of the separate mixtures are shown in Table 14. From Table 14 can be deducted that the mixtures had the following titers: mixture A, 64; mixture B, 256; mixture C, >1024; mixture D, 256; and mixture E, 16. From this can be concluded that, when both antibodies were tested in combination (mixtures B-D), the neutralizing titers were higher than those for antibody CR03-014 and CR03-022 individually. Together, these data indicate that antibodies CR03-014 and CR03-022 exhibit synergistic SARS-CoV neutralizing activity.

In yet another embodiment the SARS-CoV neutralization assay showing synergy between the anti-SARS-CoV antibodies was

performed on FRhK-4 cells (ATCC CRL-1688) as follows. The SARS-CoV strain called HK-39849 (GenBank accession number AY278491) was used in a titer of 2×10^3 TCID₅₀/ml as calculated according to the method of Reed and Muench known to the average skilled person. The human anti-SARS-CoV antibodies were screened by serially 1.46-fold-dilution in maintenance medium (MM) (1% w/v FCS in MEM with antibiotic) starting at a concentration of 200 µg/ml (dilution range 200 - 6.7 µg/ml) in duplo. Four different compositions were tested: antibody CR03-014 individually, antibody CR03-022 individually, control IgG1 antibody, and antibodies CR03-014 and CR03-022 in combination (start concentration 200 µg/ml of each antibody). 110 µl of virus suspension was mixed with 110 µl of the respective recombinant human anti-SARS-CoV antibody dilution and incubated for one hour at 37°C. 100 µl of this suspension was then pipetted two times in duplicate into 96-well plates containing an 80% confluent monolayer of FRhK-4 cells in 100 µl MM. The FRhK-4 cells were cultured at 37°C and observed after 3-4 days for the development of CPE. CPE was compared to the positive control (virus inoculated cells) and negative controls (mock-inoculated cells or cells incubated with recombinant antibody only). The complete absence of CPE in an individual cell culture was defined as protection (= 100% titer reduction). The concept of the combination index (CI) was used to quantitate synergistic effects as described previously (Chou and Talalay, 1984). According to the concept a combination of agents that produce an additive effect the sum of the ratios of their concentrations in the mixture (cmixt) to the concentrations of agents that individually have the same effect as the mixture (ceffect) is 1. This sum is the CI. When this sum is lower than 1, the agents act in synergy.

For a two-component system, as in the present study, CI is calculated as follows:

$$\frac{C1_{mixt}}{C1_{eff}} + \frac{C2_{mixt}}{C2_{eff}} = 1$$

C1mixt is the concentration of the first component in the mixture which leads to a certain level of inhibition (f),
 10 cleffect is that concentration of the first component which alone (in the absence of the second component) will result in the same inhibitory effect as the mixture of the two components, and c2mixt and c2effect are the corresponding concentrations for the second component.

15 To determine the CI for antibodies CR03-014 and CR03-022 the neutralization assay was performed as described above. Complete neutralization of 100 TCID₅₀ of strain HKU39849 was reached at 7.6 µg/ml for CR03-014, 50.0 µg/ml for CR03-022 and 2.4 µg/ml of each antibody when added in combination. This
 20 outcome results in a CI of 2.4/7.6 + 2.4/50.0= 0.36. Fifty percent neutralization was achieved at 5.9 µg/ml for CR03-014, 30.2 µg/ml for CR03-022 and 1.7 µg/ml of each antibody when added in combination. This results in a CI of 1.7/5.9 + 1.7/30.2= 0.34. Thus, at both 50 and 100% neutralization CI
 25 values lower than 1 were obtained for a mixture of CR03-014 and CR03-022, which indicates that both antibodies act in synergy against SARS-CoV.

Example 7

30 *Binding of anti-SARS antibodies to SARS-CoV, SARS-CoV spike protein and fragments thereof.*

An ELISA to detect binding of anti-SARS antibodies to SARS-CoV was performed as follows. Wells of ELISA plates were coated overnight with UV-inactivated SARS-CoV preparation in 50 mM bicarbonate buffer pH 9.6. The wells of the plates were washed three times with PBS containing 0.05% Tween and blocked for 2 hours at 37°C with PBS containing 1% BSA. Next, the antibodies diluted in PBS containing 1% BSA were incubated for 1 hour at room temperature. The wells were washed three times with PBS containing 0.05% Tween and incubated for 1 hour at room temperature using a murine anti-Hu-IgG HRP conjugate. Development was done with O-phenylenediamine substrate, the reaction was stopped by the addition of 1 M H₂SO₄ and the absorbance was measured at 492 nm. As shown in Figure 2 antibodies CR03-014 and CR03-022 were both capable of binding an inactivated SARS-CoV preparation in ELISA in contrast to a negative control IgG which was directed against an irrelevant antigen.

To detect the target of the antibodies CR03-014 and CR03-022 another binding assay was used. Single chain phage antibodies SC03-014 and SC03-022 were analyzed for their ability to bind HEK293T cells that recombinantly express proteins of SARS-CoV. To this end HEK293T cells were transfected with a plasmid carrying a cDNA sequence encoding the spike (S) protein from SARS-CoV strain Frankfurt 1 or with control vector. For flow cytometry analysis, single-chain phage antibodies were first blocked in an equal volume of 4% PBS-M for 15 minutes at 4°C prior to the staining of the transfected HEK293T cells. The blocked phage antibodies were added to mock transfected HEK293T cells and HEK293T cells transfected with the SARS-CoV S protein. The binding of the single chain phage antibodies to the cells was visualized using a biotinylated anti-M13 antibody (Santa Cruz

Biotechnology) followed by streptavidin-phycoerythrin (Caltag). As shown in Figure 3, the single chain phage antibodies SC03-014 and SC03-022 were capable of binding spike transfected HEK293T cells, whereas no binding to mock transfected HEK293T cells was observed. A control single chain phage antibody did neither recognize the spike transfected HEK293T cells nor the mock transfected HEK293T cells. These data suggest that both antibodies are directed against the S protein of SARS-CoV.

To further localize the binding sites of the antibodies within the S protein, an assay was performed wherein the antibodies were analyzed for their ability to bind to portions of the S protein of SARS-CoV. The nucleotide and amino acid sequence of the S protein is shown in SEQ ID NO:114 and SEQ ID NO:115, respectively. DNA coding for the N-terminal 565 amino acids (portion called S565) was cloned as a *KpnI*-*Bam*HI fragment in pAdapt (Havenga et al., 2001) that was modified by insertion of the polylinker of the vector called pcDNA3.1/myc-His C (Invitrogen) (vector called pAdapt/myc-His C). A fragment corresponding to amino acid residues 318-510 of the S protein (portion called S318-510) was amplified on S gene cDNA using the oligonucleotide primers: *EcoRI*sipikeFor318 5'-cctggaattctccatggccaacatcaccaacc-3' (SEQ ID NO:116) and *XbaI*sipikeRev510 5'-gaagggccctctagacacggtggcagg-3' (SEQ ID NO:117). The resulting fragment was digested with *EcoRI*-*XbaI* and cloned into pHAVT20/myc His A to yield pHAVT20/myc-His A S318-510. In this vector expression of fragment S318-510 fused to the HAVT20 leader sequence was under control of the human, full-length, immediate-early CMV promoter. DNA transfections were performed in HEK293T cells for transient expression using standard techniques. The S protein derived fragments were used directly from culture supernatant or were purified from

culture supernatant using Ni-NTA (Qiagen). An ELISA to evaluate binding of antibodies to the S protein derived fragments was performed as follows. Wells of ELISA plates were coated overnight with 5 µg/ml anti-myc antibody in 50 mM bicarbonate buffer pH 9.6. The wells of the plates were washed three times with PBS containing 0.05% Tween and blocked for 2 hours at 37°C with PBS containing 1% BSA. The wells coated with anti-myc antibody were incubated with the myc-tagged fragments S565 or S318-510 diluted in PBS containing 1% BSA for 1 hour at room temperature. The wells were washed three times with PBS containing 0.05% Tween. Next, the antibodies CR03-014, CR03-022 or control antibody diluted in PBS containing 1% BSA were incubated for 1 hour at room temperature. Detection of bound antibody was performed as described *supra*. As shown in Figure 4, antibodies CR03-014 and CR03-022 were both capable of binding to the S565 and S318-510 fragment, but not to an irrelevant control myc-tagged antigen. A control antibody did not bind any of the fragments.

In order to rank the affinities of the antibodies for binding to the S565 fragment, a titration of IgG concentration was performed (by diluting the antibodies in PBS containing 1% ELK), followed by an ELISA as described above. Titration of the monoclonal antibodies showed that CR03-014 and CR03-022 bound S565 with approximately similar affinities (see Figure 5). To investigate changes of affinities as a possible mechanism of synergy, the K_D for CR03-014 and CR03-022 binding sequentially or simultaneously to recombinant receptor binding domain fragment S318-510 was investigated by means of BIAcore analysis. Surface plasmon resonance analyses were performed at 25°C on a BIAcore3000™. CM5 sensorchips and running buffer HBS-EP were from Biacore AB (Uppsala Sweden). Recombinant S318-510 fragment was immobilized to CM5 chips using an amine

coupling procedure resulting in a response level of approximately 1,000 resonance units (RU). Kinetic analysis was performed to determine the association rate (k_a), dissociation rate (k_d) constants and the affinity (K_D) of the monoclonal antibodies. Therefore, a concentration series of 0.4 to 250 nM IgG was prepared using two-fold dilutions in HBS-EP. Samples were injected in duplicate at a flow rate of 30 μ l/min (injection time = 2 min, dissociation time = 5 min). The sensor chip surface was regenerated with a pulse of 5 μ l 5 nM NaOH. Biacore evaluation software (BIAevaluation, July 2001) was used to fit the association and dissociation curves of all concentrations injected. The individual K_D for CR03-014 and CR03-022 was determined as 16.3 nM and 0.125 nM, respectively, the K_D for the antibodies binding simultaneously as 5.71 nM and for binding of CR03-014 to CR03-022 saturated S318-510 as 14.8 nM. Compared to the dose reduction indices of 3 and 20 for CR03-014 and CR03-022, respectively, neither simultaneous nor sequential binding of the monoclonal antibodies resulted in changes of K_D which could explain their synergistic neutralizing action through cooperative binding.

To further explore the antibody binding sites within the S protein, a competition ELISA on immobilized S318-510 fragment was performed. Captured S318-510 was incubated with non-saturating amounts of biotinylated antibody without competing antibody or in the presence of 1, 5, and 25 μ g/ml of competing antibody (antibody CR03-014, CR03-022 or control antibody). Bound biotinylated antibody was detected with streptavidin-conjugated-HRP (BD Pharmingen) and developed as described above. Figure 6A shows that binding of monoclonal antibody CR03-014 was unaffected in the presence of excess unlabeled monoclonal antibody control or antibody CR03-022. Figure 6B shows that binding of antibody CR03-022 was

unaffected in the presence of excess unlabeled monoclonal antibody control or antibody CR03-014. As expected, binding of both biotinylated CR03-014 and CR03-022 was effectively reduced by their unlabeled counterparts (see Figures 6A and B). These results demonstrate that the antibodies CR03-014 and CR03-022 do not compete with each other for binding to the S318-510 fragment and recognize different/distinct, non-competing epitopes.

To confirm these findings, an antibody sandwich ELISA was performed. Antibodies CR03-014 and CR03-022 were coated overnight to microtiter wells at 5 µg/ml in 50 mM bicarbonate buffer pH 9.6. Capture of the S318-510 fragment, binding of biotinylated antibodies and subsequent development of the ELISA reaction was performed as described *supra*. Figure 7 indicates that CR03-014-captured S318-510 could be bound by biotinylated CR03-022, but not by CR03-014. CR03-022-captured S318-510 could only be bound by biotinylated CR03-014 and not CR03-022. This indicates that CR03-014 and CR03-022 are able to bind simultaneously to different epitopes within the S derived fragment S318-510 and furthermore indicates that both antibodies bind to different non-competing epitopes.

Example 8

Construction and evaluation of binding of the monoclonal anti-SARS-CoV antibodies to variant S318-510 fragments.

The diversity within the region 318-510 of the S protein was determined as follows. A list containing more than 146 genomes or genes encoding complete human SARS-CoV or fragments thereof was compiled. In 114 cases, an open reading frame encoding for full-length spike (S) protein was identified. Alignment of the spike amino acid residues 318-510 revealed 30 spike proteins, in which the region 318-510 was not identical

to that of the spike protein of strain Frankfurt 1 (see Genbank accession number AY291315), which was used herein as wild-type. The mutations, strains and Genbank numbers are depicted in Table 15. To investigate if CR03-014 and CR03-022 bound the S protein of all currently known human SARS-CoV isolates, eight recombinant spike 318-510 fragments (variants A-F and variants H and I) harbouring the different amino acid substitutions as shown in Table 15 were generated. In addition, a 318-510 fragment (variant G) corresponding to the sequence of four animal SARS-like CoVs (Genbank accession numbers AY304486 - AY304489; see also Table 1, SARS-CoV SZ3, SZ13, SZ16 and SZ1, respectively) was generated. The four SARS-like CoVs, which were isolated from raccoon dogs and palm civet cats, contain the amino acid substitutions K344R, F360S, N479K and T487S (see Guan *et al.* 2003). To this end, the above substitutions were introduced in the pHAVT20/myc-His A S318-510 vector using the Stratagene's QuikChange[®] II site-directed mutagenesis kit according to the manufacturer's instructions. In case a sequence contained multiple amino acid substitutions, the process of mutagenesis, sequence analysis and confirmation was repeated for every single substitution. To rule out the introduction of additional mutations in the plasmid outside the gene of interest, the mutated (592 bp *EcoRI-XbaI*) fragment was recloned in *EcoRI-XbaI* cut pHAVT20/myc-His A. The resulting plasmids were transfected into HEK293T cells, and binding of CR03-014 and CR03-022 was evaluated by means of ELISA as described *supra*. As shown in Figure 8, CR03-014 was capable of binding to variants A-E and variants H and I to a similar extent as to the wild-type fragment. Binding of CR03-014 to variant F (N479S substitution) was substantially lower than binding to the other fragments. No binding of CR03-014 to fragment G (K344R,

*Trade-mark

F360S, N479K and T487S substitutions) was observed. Antibody CR03-022 was capable of binding all variant S318-510 fragments to a similar extent as the wild-type (non-mutated) S318-510 fragment. Together this indicates that residue N479 is
5 involved in binding of CR03-014, either directly by being part of the binding site of CR03-014 or indirectly by being important for the correct conformation of the binding site of CR03-014 within the S protein. Since, antibody CR03-022 is capable of binding to recombinant fragments composed of amino
10 acid residues 318-510 of all human SARS-CoV isolates (as described in Table 15) and in addition is also capable of binding to animal SARS-like CoV, CR03-022 is suitable for treatment and/or protection against SARS-CoV isolates in general.

15 Particularly suitable for treatment and/or protection against human SARS-CoV isolates is a combination/cocktail comprising both antibodies, CR03-014 and CR03-022, as both antibodies are capable of specifically binding to human SARS-CoV and the antibodies act synergistically in neutralizing
20 human SARS-CoV. In other words, the combination/cocktail of CR03-014 and CR03-022 comprises synergistic human SARS-CoV neutralizing activity. An additional advantage of such a combination/cocktail is its capability of neutralizing human SARS-CoV as well as animal SARS-like CoV.

Example 9

Generation of CR03-014 and CR03-022 escape viruses of SARS-CoV

To further elucidate the epitopes recognized by the human monoclonal antibodies CR03-014 and CR03-022, escape viruses of
30 CR03-014 and CR03-022 were generated. The process for generating escape viruses of CR03-014 is given *infra*. The process for generating escape viruses of CR03-022 was

identical with the proviso that 60 µg/ml antibody instead of 20 µg/ml was used in all respective steps. Serial dilutions (0.5 ml) of SARS-CoV strain HKU 39849 (dilutions ranging from 10^{-1} – 10^{-8}) were incubated with a constant amount (20 µg/ml giving a ~3 log reduction of TCID₅₀/ml) of antibody CR03-014 (0.5 ml) for 1 hour at 37°C/5% CO₂ before addition to wells containing FRhK-4 cells. The virus was allowed to attach to the cells for 1 hour at 37°C/5% CO₂, then removed and cells were washed twice with medium. Finally, cells were incubated for 2 days in the presence of selecting antibody CR03-014 at 20 µg/ml in 0.5 ml medium. Then, medium of wells with highest virus dilution showing CPE (cytopathic effect) containing potential escape viruses was harvested and stored at 4°C until further use. Subsequently, virus samples were freeze/thawed once and serial dilutions were prepared in medium. Virus dilutions were added to wells containing FRhK-4 cells and incubated for 1-2 hours at 37°C/5% CO₂ in the presence of CR03-014 at 20 µg/ml. Wells were then overlayed with agarose containing CR03-014 at 20 µg/ml and incubated for 3-5 days at 37°C/5% CO₂. Individual escape virus plaques were picked using a Pasteur pipet, freeze/thawed once, and the escape viruses were amplified on FRhK-4 cells. To analyse the escape viruses the following experiments were performed.

Firstly, to identify possible mutations in the SARS-CoV spike protein of each of the escape viruses the nucleotide sequence of the SARS-CoV spike open reading frame (ORF) was determined. Viral RNA of each of the escape viruses and wild-type SARS-CoV virus was isolated and converted into cDNA by standard RT-PCR. Subsequently, the cDNA was used for nucleotide sequencing of the SARS-CoV spike ORF in order to identify mutations. Figure 9 shows the results of the

sequencing data for the five E014 escape viruses obtained. All escape viruses contained a nucleotide mutation at position 1385 (C to T), which resulted in an amino acid mutation P to L at position 462 in the spike protein. Apparently, P462L
5 resulted in loss of epitope recognition and, subsequently, loss of neutralization of SARS-CoV by CR03-014. This indicates that next to amino acid 479, also amino acid 462 is involved in the binding of CR03-014 to the S protein of SARS-CoV. The results of the sequencing data for the five E022 escape
10 viruses obtained were as follows. Four out of five escape viruses contained a nucleotide mutation at position 2588 (C to T), which resulted in an amino acid mutation T to I at position 863 in the spike protein.

Secondly, the neutralization index (NI) was determined
15 for each of the E014 and E022 escape viruses. A virus was defined as an escape variant, if the neutralization index was <2.5 logs. The process of determining the NI is given below for E014 escape viruses. The process was identical for E022 escape viruses with the proviso that 60 µg/ml instead of 20
20 µg/ml monoclonal antibody was used in all respective steps. The neutralization index was determined by subtracting the number of infectious virus particles (in TCID₅₀/ml) produced in FRhK-4 cell cultures infected with virus plus monoclonal antibody (20 µg/ml) from the number of infectious virus particles (in
25 TCID₅₀/ml) produced in FRhK-4 cell cultures infected with virus alone ([log TCID₅₀/ml virus in absence of monoclonal antibody minus log TCID₅₀/ml virus in presence of monoclonal antibody]). An index lower than 2.5 logs was considered as evidence of escape. Therefore, each escape virus and wild-type
30 SARS-CoV (100 TCID₅₀) was incubated for 1 hour at 37°C/5% CO₂ with 20 µg/ml of CR03-014 before addition to FRhK-4 cells. The

virus was allowed to attach to the cells for 1 hour at 37°C/5% CO₂ after which the inoculum was removed and cells were washed twice with medium before being replenished with medium containing 20 µg/ml of CR03-014. After a 2 day incubation at 37°C/5% CO₂ the medium was harvested and the TCID₅₀/ml of each virus was determined. As shown in Table 16, the concentration of antibody used to determine the NI resulted in a ~3 log reduction of virus titer when performed on the wild-type SARS-CoV virus. Thus, wild type SARS-CoV was neutralized by CR03-014 as judged by the NI of 3.3. In contrast, the NI for each escape virus was <2.5 and thus each of the escape viruses was no longer neutralized by CR03-014. As judged by the NI of 3.3, wild-type SARS-CoV virus was also neutralized by CR03-022 (see Table 17). The NI for each E022 escape virus was >2.5 and thus it was concluded that each of the escape viruses was still neutralized by CR03-022. The amino acid substitution in four of the five E022 escape viruses apparently does not play a role in neutralization of SARS-CoV by CR03-022. It might have been induced non-specifically during the course of the experiment. This agrees with finding by Poon *et al.* (2005) who observed the mutation at position 863 (T to I) when SARS-CoV was passaged multiple times in FRhK-4 cells. The neutralizing epitope of CR03-022 could not be determined by means of generating escape viruses. This may be caused by the functional constraints of the binding region on the S protein. A mutation occurring in this region may be detrimental to the stability of the virus and could therefore not be isolated in the experiments described above.

In order to confirm lack of binding of antibody CR03-014 to the escape variant, a recombinant S318-510 fragment harbouring the P to L substitution at position 462 was

constructed essentially as described *supra*. DNA transfection of the resulting plasmid was performed in HEK293T cells for transient expression and the fragments were directly used from culture supernatant. The ELISA was performed as described

5 *supra*. Briefly, the fragments were captured on anti-myc coated microtiter wells. Subsequently, antibodies CR03-014 and CR03-022 were added and binding of the antibodies was detected using an anti-human IgG HRP-conjugate. As shown in Figure 10, antibody CR03-014 was not able to bind the S318-510 fragment

10 carrying a P to L substitution at position 462. Binding of CR03-022 was not affected by this amino acid substitution. This further indicates that antibody CR03-022 is capable of binding to a different/distinct, non-competing epitope on the S protein and suggests that CR03-022 might be used to

15 compensate potential lack of neutralization of SARS-CoV variants by CR03-014.

Furthermore, cross-neutralization assays were performed on E14 escape viruses. Table 18 clearly shows that CR03-022 neutralized the E14 escape viruses to a similar level as wild-

20 type virus further illustrating that CR03-022 binds to a different epitope compared to CR03-014, which no longer neutralized the E14 escape viruses. This result is in agreement with the ELISA data shown in Figure 10. The reverse experiment could not be performed as escape viruses of CR03-

25 022 could not be generated. From the above can be concluded that the combination of CR03-014 and CR03-022 in a cocktail prevents the escape of neutralization-resistant SARS-CoV variants and is therefore an ideal immunoglobulin preparation for SARS-CoV prophylaxis and therapy.

Example 10

Assessment of potential enhancement of SARS-CoV infection in human macrophages by the human anti-SARS monoclonal antibody CR03-014

5 It is known that in certain diseases caused by
coronaviruses prior immunity or passive antibody increases the
severity of the disease. In feline infectious peritonitis, the
macrophage is the main target cell for virus replication and
anti-viral antibodies enhance replication of the virus in
10 macrophage cultures *in vitro*. The macrophage is also a
prominent cell seen in the cell infiltrates of lungs of
patients dying of SARS-CoV (Nicholls *et al.* 2003). This has
led to concern whether antibody dependent enhancement (ADE)
may be relevant in the pathogenesis of SARS-CoV. To
15 investigate this it was tested whether ADE occurred when
macrophages were infected with SARS-CoV in the presence of the
neutralizing anti-SARS-CoV monoclonal antibody CR03-014, the
non-neutralizing anti-SARS-CoV monoclonal antibody CR-03-015,
the monoclonal antibody called CR-JA (an monoclonal antibody
20 against rabies which is used herein as a control antibody),
convalescent serum from an individual exposed to SARS-CoV and
serum from a healthy individual.

Human peripheral blood mononuclear cells (PBMCs) were
obtained from leukocyte-rich buffy coats of healthy blood
25 donors (The Hong Kong Red Cross Blood Transfusion Service,
Hong Kong). The PBMCs were separated by Ficoll-Paque* gradient
centrifugation (Pharmacia Biotech, Uppsala, Sweden). To
isolate monocytes 2×10^7 PBMCs were allowed to adhere onto
petridishes (Greiner, Frickenhausen, Germany) for 1 hour in
30 RPMI 1640 medium supplemented with 20 mM HEPES, 2 mM
glutamine, 0.6 µg/ml penicillin, and 60 µg/ml streptomycin and
5% heat-inactivated autologous plasma. After washing with

*Trade-mark

medium, the adherent monocytes were detached by pipeting and re-seeded into 24 well plates at a density of 2×10^5 cells per well in supplemented RPMI 1640 medium.

To check the purity of the monocyte preparations monocytes were also seeded and allowed to adhere onto glass coverslips. The purity of the monocytes on the glass coverslips was confirmed by staining with a CD14 R-phycoerythrin (R-PE)-conjugated mouse anti-human monoclonal antibody (BD Biosciences, San Diego, U.S.A.).

Medium in the monocyte cultures was replaced every 2-3 days and the cells were allowed to differentiate for 14 days *in vitro*. Differentiation of monocytes into macrophages was confirmed by the typical morphology of macrophages. The obtained primary human monocyte-derived macrophages were used in further experiments. Two days prior to the ADE experiments, the supplemented RPMI 1640 medium was exchanged into Macrophage Serum Free medium (SFM) (Invitrogen, Carlsbad, CA, U.S.A.).

To investigate the effect of sub-neutralizing doses of antibody on viral infection in macrophages, 300 μ l of serial 10-fold dilutions in MM medium (MEM including 1% FCS and 0.6 μ g/ml penicillin, and 60 μ g/ml streptomycin) of the monoclonal antibodies CR03-014, CR03-015 and CR-JA, convalescent serum from a SARS-CoV exposed individual and serum from a healthy individual was mixed with 300 μ l of SARS-CoV. MM medium mixed with SARS-CoV served as the virus control. The virus/monoclonal antibody mixtures and virus/serum mixtures were incubated for one hour at 37°C. Then, 250 μ l of the mixtures was added to duplicate wells containing macrophages. After one hour of virus adsorption at 37°C, the virus inoculum was removed, infected cells were washed with macrophage SFM culture medium and incubated in macrophage SFM medium

supplemented with 0.6 µg/ml penicillin and 60 µg/ml streptomycin. Samples of the culture supernatants were collected at days 0, 1, 2, 3, 5, and 7 post-infection and stored at -70°C for virus titration experiments. SARS-CoV was
5 titrated and the TCID₅₀ determined essentially as described supra.

To detect virus inside the macrophages (due to abortive infection) and to detect potential transcription of SARS-CoV RNA inside the macrophages RNA was isolated from infected
10 macrophages at 3, 6, and 24 hours post-infection using the RNeasy* Mini kit (Qiagen) according to the manufacturer's instructions. Reverse transcription with oligo-dT primers was performed by using Superscript* II reverse transcriptase (Invitrogen) according to the manufacturer's instructions.

15 Complementary DNA was generated with 10 µl of RNA, and reverse-transcribed by 200 U of Superscript II reverse transcriptase (Invitrogen) in a 20 µl reaction containing 25 ng oligo-dT₁₂₋₁₈ primer, 10 mM dithiothreitol, and 0.5 mM deoxynucleotide triphosphates. Reactions were incubated at
20 42°C for 50 minutes, followed by a heat inactivation step (72°C for 15 minutes). The reaction mix was diluted 10 times by the addition of 180 µl buffer AE (Qiagen) and stored at -20°C.

Real time quantitative PCR was performed using FastStart
25 DNA Master SYBR Green I fluorescence reaction (Roche). 5 µl of diluted complementary DNA was amplified in a 20 µl reaction containing 4 mM of MgCl₂, 0.5 mM of forward primer (Actin-LF: CCCAAGGCCAACCGCGAGAAGAT (SEQ ID NO:122)), and 0.5 mM of reverse primer (Actin-LR: GTCCCGGCCAGCCAGGTCCAG (SEQ ID
30 NO:123)). Reactions were performed in a LightCycler* (Roche) with the following conditions: 10 minutes at 95°C, followed by 40 cycles of 95°C for 0 seconds, 66°C for 5 seconds, and 72°C

*Trade-mark

for 9 seconds. Plasmids containing the target sequence were used as positive controls. Fluorescence signals from these reactions were captured at the end of the extension step in each cycle (87°C). To determine the specificity of the assay, 5 PCR products were subjected to melting curve analysis at the end of the assay (65 to 95°C; 2°C/second). Reverse transcription with sense (negative strand detection) or anti-sense (positive strand detection) primers to the polymerase gene of SARS-CoV was achieved by using Superscript II reverse transcriptase (Invitrogen) according to manufacturer's 10 instructions. Complementary DNA was generated with 5 µL of RNA, and reverse-transcribed by 200 U of Superscript II reverse transcriptase (Invitrogen) in a 20 µl reaction containing 0.1 µM gene specific primer, 10 mM dithiothreitol, 15 and 0.5 mM deoxynucleotide triphosphates. Reactions were incubated at 42°C for 50 minutes, followed by a heat inactivation step (72°C for 15 minutes). The reaction was diluted 10 times by the addition of 180 µL buffer AE (Qiagen) and stored at -20°C. 2 µl of diluted complementary DNA was 20 amplified in 20 µl containing 3.5 mM of MgCl₂, 0.25 µM of forward primer (coro3: 5'-TACACACCTCAGCGTTG-3' (SEQ ID NO:124)), and 0.25 µM of reverse primer (coro4: 5'-CACGAACGTGACGAAT-3' (SEQ ID NO:125)). Reactions were performed in a LightCycler (Roche) with the following conditions: 10 min 25 at 95°C, followed by 50 cycles of 95°C for 10 seconds, 60°C for 5 seconds, and 72°C for 9 seconds. Plasmids containing the target sequence were used as positive controls. Fluorescence signals from these reactions were captured at the end of the extension step in each cycle. To determine the specificity of 30 the assay, PCR products were subjected to melting curve analysis at the end of the assay (65 to 95°C; 0.1°C/seconds).

Data for viral RNA were normalised for RNA levels of β -actin housekeeping gene.

After infection of macrophages with SARS-CoV, the cells were monitored daily under the microscope. Ten days after
5 infection with SARS-CoV no detectable cytopathic effect was detected, nor was the abortive infection of macrophages converted into a productive infection. Pre-incubating of SARS-CoV with different concentrations of the monoclonal antibodies CR03-014, CR03-015, CR-JA, convalescent serum or serum from a
10 healthy individual, prior to infection of macrophages did not change this out-come.

Titration of aliquots of supernatant taken from infected macrophage culture at days 0, 1, 2, 3, 5, and 7 days post-infection on FRhk-4 cells revealed no evidence of significant
15 enhancement of SARS-CoV replication in macrophages by the monoclonal antibodies and sera tested (data not shown).

In addition, the effect of antibodies on the (abortive) infection of macrophages by SARS-CoV and the transcription of SARS-CoV genes within the macrophages was measured on the
20 molecular level. To this end, total RNA was extracted from the infected macrophages at various time points post-infection as described *supra*. Subsequently, total RNA was analyzed for SARS-CoV viral positive strand RNA and viral negative strand RNA transcripts using real-time RT-PCR. The SARS-CoV RNA
25 levels were normalized for the levels of β -actin mRNA. The results show that positive strand SARS-CoV RNA was detected in all macrophage cultures that were incubated with SARS-CoV, which confirms the abortive infection of macrophages by SARS-CoV. The levels of positive strand RNA observed in macrophage
30 cultures infected with SARS-CoV in the presence of anti-SARS-CoV monoclonal antibodies CR03-014 or CR03-015 or convalescent serum were not significantly higher than in macrophage

cultures infected with SARS-CoV in the presence of control monoclonal antibody CR-JA or serum from a healthy individual or in the absence of monoclonal antibody or serum (data not shown).

5 Furthermore, the presence of negative strand RNA which is indicative for SARS-CoV gene transcriptional activity after infection of macrophages by SARS-CoV was measured. Again no correlation between the levels of negative strand RNA and the presence or absence of anti-SARS-CoV antibodies was observed
10 (data not shown).

 Together, the experiments assaying infectious virus yield and virus related RNA levels inside macrophages showed that there was no antibody dependent enhancement of SARS-CoV replication in human macrophages by anti-SARS-CoV monoclonal
15 antibodies CR03-014 and CR03-015 and anti-SARS-CoV antibodies present in convalescent serum from a SARS patient.

Example 11

*Identification of epitopes recognized by recombinant human
20 anti-SARS-CoV antibodies by PEPSCAN-ELISA*

 15-mer linear and looped/cyclic peptides were synthesized from proteins of SARS-CoV and screened using credit-card format mini-PEPSCAN cards (455 peptide formats/card) as described previously (see *inter alia* WO 84/03564, WO 93/09872,
25 Slootstra *et al.* 1996). All peptides were acetylated at the amino terminus. In short, series of overlapping peptides, which were either in linear form or in looped/cyclic form, of the spike protein of SARS-CoV Urbani (the protein-id of the surface spike glycoprotein in the EMBL-database is AAP13441),
30 was produced and tested for binding to the recombinant human anti-SARS-CoV antibodies of the invention by means of PEPSCAN analysis.

Because the Urbani spike protein indicated above was also found in identical or highly homologous form in other SARS-CoV strains, the antigenic peptides found in the analysis method may not only be used for detection of the SARS-CoV strain
5 Urbani and the prevention and/or treatment of a condition resulting from the SARS-CoV strain Urbani, but may also be useful in detecting SARS-CoV in general and preventing and/or treating a condition resulting from SARS-CoV in general. The protein-id of the surface spike glycoprotein of for instance
10 the SARS-CoV strains TOR2, Frankfurt 1 and HSR 1 in the EMBL-database is AAP41037, AAP33697 and AAP72986. The accession number in the EMBL-database of the complete genome of the strains TOR2, Frankfurt 1 and HSR 1 is AY274119, AY291315 and AY323977, respectively. Under these accession numbers the
15 amino acid sequence of the other (potential) proteins of these strains can be found.

In all looped peptides position-2 and position-14 were replaced by a cysteine (acetyl-XCXXXXXXXXXXCX-minicard). If other cysteines besides the cysteines at position-2 and
20 position-14 were present in a prepared peptide, the other cysteines were replaced by an alanine. The looped peptides were synthesized using standard Fmoc-chemistry and deprotected using trifluoric acid with scavengers. Subsequently, the deprotected peptides were reacted on the cards with an 0.5 mM
25 solution of 1,3-bis(bromomethyl)benzene in ammonium bicarbonate (20 mM, pH 7.9/acetonitril (1:1 (v/v))). The cards were gently shaken in the solution for 30-60 minutes, while completely covered in the solution. Finally, the cards were washed extensively with excess of H₂O and sonicated in
30 disrupt-buffer containing 1% SDS/0.1% beta-mercaptoethanol in PBS (pH 7.2) at 70°C for 30 minutes, followed by sonication in H₂O for another 45 minutes.

Recombinant human anti-SARS-CoV antibodies CR03-014 and CR03-022 were tested for binding to each linear and looped peptide in a PEPSCAN-based enzyme-linked immuno assay (ELISA). The 455-well creditcard-format polypropylene cards, containing the covalently linked peptides, were incubated with the antibodies (1-10 µg/ml; diluted in blocking solution which contains 5% horse-serum (v/v) and 5% ovalbumin (w/v)) (4°C, overnight). After washing, the peptides were incubated with anti-human antibody peroxidase (dilution 1/1000) (1 hour, 25°C), and subsequently, after washing the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 µl/ml 3% H₂O₂ were added. Controls (for linear and looped) were incubated with anti-human antibody peroxidase only. After 1 hour the colour development was measured. The colour development of the ELISA was quantified with a CCD-camera and an image processing system. The set-up consisted of a CCD-camera and a 55 mm lens (Sony CCD Video Camera XC-77RR, Nikon micro-nikkor 55 mm f/2.8 lens), a camera adaptor (Sony Camera adaptor DC-77RR) and the Image Processing Software package Optimas, version 6.5 (Media Cybernetics, Silver Spring, MD 20910, U.S.A.). Optimas runs on a pentium II computer system.

The recombinant human anti-SARS-CoV-antibodies CR03-014 and CR03-022 were tested for binding to the 15-mer linear and looped/cyclic peptides synthesized as described supra. Relevant binding of a peptide to a recombinant human anti-SARS-CoV antibody was calculated as follows. The average OD-value for each antibody was calculated for the respective proteins (sum of OD-values of all peptides/total number of peptides). Next, the standard deviation of this average was calculated. The standard deviation was multiplied by 2 and the obtained value was added to the average value to obtain the

correction factor. The OD-value of each peptide was then divided by this correction factor. If a value of 0.9 or higher was found, then relevant binding was considered to be present between the specific peptide and the respective antibody.

- 5 Particularly interesting appear to be domains comprising several reactive peptides, *i.e.* domains comprising consecutive peptides, wherein at least most of the peptides in the domains are reactive with the antibody.

Monoclonal antibody CR03-014 did not appear to react
10 specifically with a peptide or domains comprising several peptides within the SARS-CoV spike protein indicating that CR03-014 may recognize a discontinuous non-linear epitope. Monoclonal antibody CR03-022 reacted with a series of looped peptides in two domains (data not shown). The domains are
15 comprised of amino acid residues 430-449 and 484-503 of the SARS-CoV spike protein and have the amino acid sequences ATSTGNINYKYRYLRHGKLR (SEQ ID NO:126) and YTTTGIGYQPYRVVLSFEL (SEQ ID NO:127), respectively. Strikingly, both domains have the motif TXTGXXXXXYR (SEQ ID NO:128) in common, indicating
20 that this motif may be crucial for the binding of antibody CR03-022 to the SARS-CoV spike protein.

Table 1. List of currently known SARS-CoV genome sequence and spike genes.

SARS isolate	Gene/genome	Genbank	FASTA
SARS coronavirus AS	SARS coronavirus AS, complete genome.	AY427439	37576845
SARS coronavirus BJ01	SARS coronavirus BJ01, complete genome.	AY278488	30275666
SARS coronavirus BJ02	SARS coronavirus BJ02, complete genome.	AY278487	31416292
SARS coronavirus BJ03	SARS coronavirus BJ03, complete genome.	AY278490	31416305
SARS coronavirus BJ04	SARS coronavirus BJ04, complete genome.	AY279354	31416306
SARS coronavirus BJ2232			
SARS coronavirus BJ302			
SARS coronavirus CUHK-AG01	SARS coronavirus CUHK-AG01, complete genome.	AY345986	33114190
SARS coronavirus CUHK-AG02	SARS coronavirus CUHK-AG02, complete genome.	AY345987	33114202
SARS coronavirus CUHK-AG03	SARS coronavirus CUHK-AG03, complete genome.	AY345988	33114214
SARS coronavirus CUHK-L2			
SARS coronavirus CUHK-Su10	SARS coronavirus CUHK-Su10, complete genome.	AY282752	38304867
SARS coronavirus CUHK-W1	SARS coronavirus CUHK-W1, complete genome.	AY278554	30027610
SARS coronavirus cw037			
SARS coronavirus cw049			
SARS coronavirus FRA	SARS coronavirus FRA, complete genome.	AY310120	33578015
SARS coronavirus Frankfurt 1	SARS coronavirus Frankfurt 1, complete genome.	AY291315	31581502
SARS coronavirus GD01	SARS coronavirus GD01, complete genome.	AY278489	31416290
SARS coronavirus GD03T0013	SARS coronavirus GD03T0013 spike glycoprotein gene, complete cds.	AY525636	41764105
SARS coronavirus GD69	SARS coronavirus GD69, complete genome.	AY313906	37960831
SARS coronavirus GZ-A	SARS coronavirus GZ-A, partial genome.	AY394977	37624320
SARS coronavirus GZ-B	SARS coronavirus GZ-B, complete genome.	AY394978	37624321
SARS coronavirus GZ-C	SARS coronavirus GZ-C, complete genome.	AY394979	37624322
SARS coronavirus GZ-D	SARS coronavirus GZ-D, partial genome.	AY394980	37624323
SARS coronavirus GZ02	SARS coronavirus GZ02, complete genome.	AY390556	41323719
SARS coronavirus GZ43	SARS coronavirus GZ43, partial genome.	AY304490	34482141
SARS coronavirus GZ50	SARS coronavirus GZ50, complete genome.	AY304495	34482146
SARS coronavirus GZ60	SARS coronavirus GZ60, partial genome.	AY304491	34482142
SARS coronavirus HB			
SARS coronavirus HGZ8L1-A	SARS coronavirus HGZ8L1-A, partial genome.	AY394981	37624324
SARS coronavirus HGZ8L1-B	SARS coronavirus HGZ8L1-B, partial genome.	AY394982	37624325
SARS coronavirus HGZ8L2	SARS coronavirus HGZ8L2, complete genome.	AY394993	37624336
SARS coronavirus HKU-36871	SARS coronavirus HKU-36871, partial genome.	AY304492	34482143
SARS coronavirus HKU-39849	SARS coronavirus HKU-39849, complete genome.	AY278491	30023963
SARS coronavirus HKU-65806	SARS coronavirus HKU-65806, partial genome.	AY304493	34482144
SARS coronavirus HKU-66078	SARS coronavirus HKU-66078, partial genome.	AY304494	34482145
SARS coronavirus Hong Kong/03/2003			

SARS coronavirus HPZ-2003			
SARS coronavirus HSR 1	SARS coronavirus HSR 1, complete genome.	AY323977	33115118
SARS coronavirus HSZ-A	SARS coronavirus HSZ-A, partial genome.	AY394984	37624327
SARS coronavirus HSZ-Bb	SARS coronavirus HSZ-Bb, complete genome.	AY394985	37624328
SARS coronavirus HSZ-Bc	SARS coronavirus HSZ-Bc, complete genome.	AY394994	37624337
SARS coronavirus HSZ-Cb	SARS coronavirus HSZ-Cb, complete genome.	AY394986	37624329
SARS coronavirus HSZ-Cc	SARS coronavirus HSZ-Cc, complete genome.	AY394995	37624338
SARS coronavirus HSZ2-A	SARS coronavirus HSZ2-A, complete genome.	AY394983	37624326
SARS coronavirus HZS2-Bb	SARS coronavirus HZS2-Bb, partial genome.	AY395004	37624347
SARS coronavirus HZS2-C	SARS coronavirus HZS2-C, complete genome.	AY394992	37624335
SARS coronavirus HZS2-D	SARS coronavirus HZS2-D, complete genome.	AY394989	37624332
SARS coronavirus HZS2-E	SARS coronavirus HZS2-E, complete genome.	AY394990	37624333
SARS coronavirus HZS2-Fb	SARS coronavirus HZS2-Fb, complete genome.	AY394987	37624330
SARS coronavirus HZS2-Fc	SARS coronavirus HZS2-Fc, complete genome.	AY394991	37624334
SARS coronavirus JMD	SARS coronavirus JMD, partial genome.	AY394988	37624331
SARS coronavirus LC1	SARS coronavirus LC1, complete genome.	AY394998	37624341
SARS coronavirus LC2	SARS coronavirus LC2, complete genome.	AY394999	37624342
SARS coronavirus LC3	SARS coronavirus LC3, complete genome.	AY395000	37624343
SARS coronavirus LC4	SARS coronavirus LC4, complete genome.	AY395001	37624344
SARS coronavirus LC5	SARS coronavirus LC5, complete genome.	AY395002	37624345
SARS coronavirus NS-1	SARS coronavirus NS-1, complete genome.	AY508724	40795744
SARS coronavirus PUMC01	SARS coronavirus PUMC01, complete genome.	AY350750	38231927
SARS coronavirus PUMC02	SARS coronavirus PUMC02, complete genome.	AY357075	38231932
SARS coronavirus PUMC03	SARS coronavirus PUMC03, complete genome.	AY357076	38231937
SARS coronavirus sf098			
SARS coronavirus sf099			
SARS coronavirus ShanghaiQXC1	SARS coronavirus ShanghaiQXC1, complete genome.	AY463059	40457433
SARS coronavirus ShanghaiQXC2	SARS coronavirus ShanghaiQXC2, complete genome.	AY463060	40457448
SARS coronavirus Shanghai LY	SARS coronavirus Shanghai LY spike glycoprotein gene, complete cds.	AY322205S3	32454341
SARS coronavirus Sin0409			
SARS coronavirus Sin2500	SARS coronavirus Sin2500, complete genome.	AY283794	30468042
SARS coronavirus Sin2677	SARS coronavirus Sin2677, complete genome.	AY283795	30468043
SARS coronavirus Sin2679	SARS coronavirus Sin2679, complete genome.	AY283796	30468044
SARS coronavirus Sin2748	SARS coronavirus Sin2748, complete genome.	AY283797	30468045
SARS coronavirus Sin2774	SARS coronavirus Sin2774, complete genome.	AY283798	37361915
SARS coronavirus Sin3408	SARS coronavirus Sin3408, complete genome	AY559083	45644998
SARS coronavirus Sin3408L	SARS coronavirus Sin3408L, complete genome	AY559097	45645024
SARS coronavirus Sin3725V	SARS coronavirus Sin3725V, complete genome	AY559087	45645004
SARS coronavirus Sin3765V	SARS coronavirus Sin3765V, complete genome	AY559084	45645000
SARS coronavirus Sin842	SARS coronavirus Sin842, complete genome	AY559081	45644994

SARS coronavirus Sin845	SARS coronavirus Sin845, complete genome	AY559093	45645019
SARS coronavirus Sin846	SARS coronavirus Sin846, complete genome	AY559094	45645021
SARS coronavirus Sin847	SARS coronavirus Sin847, complete genome	AY559095	45645022
SARS coronavirus Sin848	SARS coronavirus Sin848, complete genome	AY559085	45645001
SARS coronavirus Sin849	SARS coronavirus Sin849, complete genome	AY559086	45645003
SARS coronavirus Sin850	SARS coronavirus Sin850, complete genome	AY559096	45645023
SARS coronavirus Sin852	SARS coronavirus Sin852, complete genome	AY559082	45644996
SARS coronavirus Sin_WNV			
SARS coronavirus Sino1-11	SARS coronavirus Sino1-11, complete genome.	AY485277	38505482
SARS coronavirus Sino3-11	SARS coronavirus Sino3-11, complete genome.	AY485278	38505491
SARS coronavirus SinP1	SARS coronavirus SinP1, complete genome	AY559088	45645007
SARS coronavirus SinP2	SARS coronavirus SinP2, complete genome	AY559089	45645010
SARS coronavirus SinP3	SARS coronavirus SinP3, complete genome	AY559090	45645013
SARS coronavirus SinP4	SARS coronavirus SinP4, complete genome	AY559091	45645016
SARS coronavirus SinP5	SARS coronavirus SinP5, complete genome	AY559092	45645017
SARS coronavirus SoD	SARS coronavirus SoD, complete genome.	AY461660	38385714
SARS coronavirus SZ1	SARS coronavirus SZ1, partial genome.	AY304489	34482140
SARS coronavirus SZ13	SARS coronavirus SZ13, partial genome.	AY304487	34482138
SARS coronavirus SZ16	SARS coronavirus SZ16, complete genome.	AY304488	34482139
SARS coronavirus SZ3	SARS coronavirus SZ3, complete genome.	AY304486	34482137
SARS coronavirus Taiwan			
SARS coronavirus Taiwan JC-2003			
SARS coronavirus Taiwan TC1	SARS coronavirus Taiwan TC1, complete genome.	AY338174	32493129
SARS coronavirus Taiwan TC2	SARS coronavirus Taiwan TC2, complete genome.	AY338175	32493130
SARS coronavirus Taiwan TC3	SARS coronavirus Taiwan TC3, complete genome.	AY348314	33188324
SARS coronavirus Tor2	SARS coronavirus TOR2, complete genome.	AY274119	30248028
SARS coronavirus TW			
SARS coronavirus TW-GD1			
SARS coronavirus TW-GD2			
SARS coronavirus TW-GD3			
SARS coronavirus TW-GD4			
SARS coronavirus TW-GD5			
SARS coronavirus TW-HP1			
SARS coronavirus TW-HP2			
SARS coronavirus TW-HP3			
SARS coronavirus TW-HP4			
SARS coronavirus TW-JC2			
SARS coronavirus TW-KC1			
SARS coronavirus TW-KC3			
SARS coronavirus TW-PH1			
SARS coronavirus TW-PH2			

SARS coronavirus TW-YM1			
SARS coronavirus TW-YM2			
SARS coronavirus TW-YM3			
SARS coronavirus TW-YM4			
SARS coronavirus TW1	SARS coronavirus TW1, complete genome.	AY291451	30698326
SARS coronavirus TW10	SARS coronavirus TW10, complete genome.	AY502923	40548873
SARS coronavirus TW11	SARS coronavirus TW11, complete genome.	AY502924	40548885
SARS coronavirus TW2	SARS coronavirus TW2, complete genome.	AY502925	40548897
SARS coronavirus TW3	SARS coronavirus TW3, complete genome.	AY502926	40548909
SARS coronavirus TW4	SARS coronavirus TW4, complete genome.	AY502927	40548921
SARS coronavirus TW5	SARS coronavirus TW5, complete genome.	AY502928	40548933
SARS coronavirus TW6	SARS coronavirus TW6, complete genome.	AY502929	40548945
SARS coronavirus TW7	SARS coronavirus TW7, complete genome.	AY502930	40548957
SARS coronavirus TW8	SARS coronavirus TW8, complete genome.	AY502931	40548969
SARS coronavirus TW9	SARS coronavirus TW9, complete genome.	AY502932	40548981
SARS coronavirus TWC	SARS coronavirus TWC, complete genome.	AY321118	31873092
SARS coronavirus TWC2	SARS coronavirus TWC2, complete genome.	AY362698	33518724
SARS coronavirus TWC3	SARS coronavirus TWC3, complete genome.	AY362699	33518725
SARS coronavirus TWH	SARS coronavirus TWH genomic RNA, complete genome.	AP006557	33411399
SARS coronavirus TWJ	SARS coronavirus TWJ genomic RNA, complete genome.	AP006558	33411414
SARS coronavirus TWK	SARS coronavirus TWK genomic RNA, complete genome.	AP006559	33411429
SARS coronavirus TWS	SARS coronavirus TWS genomic RNA, complete genome.	AP006560	33411444
SARS coronavirus TWY	SARS coronavirus TWY genomic RNA, complete genome.	AP006561	33411459
SARS coronavirus Urbani	SARS coronavirus Urbani, complete genome.	AY278741	30027617
SARS coronavirus Vietnam			
SARS coronavirus WHU	SARS coronavirus WHU, complete genome.	AY394850	40795428
SARS coronavirus xw002			
SARS coronavirus ZJ01	SARS coronavirus ZJ01, complete genome.	AY297028	30910859
SARS coronavirus ZMY 1	SARS coronavirus ZMY 1, complete genome.	AY351680	33304219
SARS coronavirus ZS-A	SARS coronavirus ZS-A, complete genome.	AY394997	37624340
SARS coronavirus ZS-B	SARS coronavirus ZS-B, complete genome.	AY394996	37624339
SARS coronavirus ZS-C	SARS coronavirus ZS-C, complete genome.	AY395003	37624346
	SARS coronavirus, TOR2 complete genome, curated	NC_004718	30271926
	SARS coronavirus ZJ01, partial genome.	AY286320	39980888
	SARS coronavirus BJ302 clone 1 spike glycoprotein gene, complete	AY429072	38016580
	SARS coronavirus BJ302 clone 2 spike glycoprotein gene, complete	AY429073	38016582
	SARS coronavirus BJ302 clone 3 spike glycoprotein gene, complete	AY429074	38016584
	SARS coronavirus BJ302 clone 4 spike glycoprotein gene, complete	AY429075	38016586
	SARS coronavirus BJ302 clone 5 spike glycoprotein gene, complete	AY429076	38016588
	SARS coronavirus BJ302 clone 6 spike glycoprotein gene, complete	AY429077	38016590

	SARS coronavirus BJ302 clone 7 spike glycoprotein gene, complete	AY429078	38016592
	SARS coronavirus BJ302 clone 8 spike glycoprotein gene, complete	AY429079	38016594

Table 2: Human IgG heavy chain variable region primers (sense) .

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVH1B/7A	5' -CAGRTGCAGCTGGTG CARTCTGG-3'	SEQ ID NO:11
HuVH1C	5' -SAGGTCCAGCTGGTR CAGTCTGG-3'	SEQ ID NO:12
HuVH2B	5' -SAGGTGCAGCTGGTG GAGTCTGG-3'	SEQ ID NO:13
HuVH3B	5' -SAGGTGCAGCTGGTG GAGTCTGG-3'	SEQ ID NO:14
HuVH3C	5' -GAGGTGCAGCTGGTG GAGWCYGG-3'	SEQ ID NO:15
HuVH4B	5' -CAGGTGCAGCTACAG CAGTGGGG-3'	SEQ ID NO:16
HuVH4C	5' -CAGSTGCAGCTGCAG GAGTCSGG-3'	SEQ ID NO:17
HuVH5B	5' -GARGTGCAGCTGGTG CAGTCTGG-3'	SEQ ID NO:18
HuVH6A	5' -CAGGTACAGCTGCAG CAGTCAGG-3'	SEQ ID NO:19

Table 3: Human IgG heavy chain J-region primers (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuJH1/2	5'-TGAGGAGACGGTGAC CAGGGTGCC-3'	SEQ ID NO:20
HuJH3	5'-TGAAGAGACGGTGAC CATTGTCCC-3'	SEQ ID NO:21
HuJH4/5	5'-TGAGGAGACGGTGAC CAGGGTTCC-3'	SEQ ID NO:22
HuJH6	5'-TGAGGAGACGGTGAC CGTGGTCCC-3'	SEQ ID NO:23

Table 4: Human IgG heavy chain variable region primers

5 extended with SfiI/NcoI restriction sites (sense) and human IgG heavy chain J-region primers extended with XhoI/BstEII restriction sites (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVH1B/7A-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGRTGCAGCTGGTGCA TCTGG-3'	SEQ ID NO:24
HuVH1C-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC SAGGTCCAGCTGGTRCAG TCTGG-3'	SEQ ID NO:25
HuVH2B-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGRTCACCTTGAAGGAG TCTGG-3'	SEQ ID NO:26

HuVH3B-NcoI	5'-GTCCTCGCAACTGCGGCC CAGCCGGCCATGGCCSAGGTG CAGCTGGTGGAGTCTGG-3'	SEQ ID NO:27
HuVH3C-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC GAGGTGCAGCTGGTGGAG WCYGG-3'	SEQ ID NO:28
HuVH4B-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGGTGCAGCTACAGCAG TGGGG-3'	SEQ ID NO:29
HuVH4C-NcoI	5'-GTCCTCGCAACTGCGGCC CAGCCGGCCATGGCCCAGSTG CAGCTGCAGGAGTCSGG-3'	SEQ ID NO:30
HuVH5B-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC GARGTGCAGCTGGTGCAG TCTGG-3'	SEQ ID NO:31
HuVH6A-NcoI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGGTACAGCTGCAGCAG TCAGG-3'	SEQ ID NO:32
HuJH1/2-XhoI	5'-GAGTCATTCTCGACTCGA GACGGTGACCAGGGTGCC-3'	SEQ ID NO:33
HuJH3-XhoI	5'-GAGTCATTCTCGACT CGAGACGGTGACCATTGT CCC-3'	SEQ ID NO:34
HuJH4/5-XhoI	5'-GAGTCATTCTCGACT CGAGACGGTGACCAGGGT TCC-3'	SEQ ID NO:35
HuJH6-XhoI	5'-GAGTCATTCTCGACTCGA	SEQ ID NO:36

	GACGGTGACCGTGGTCCC-3'	
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Table 5: Human lambda chain variable region primers (sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVλ1A	5'-CAGTCTGTGCTGACT CAGCCACC-3'	SEQ ID NO:37
HuVλ1B	5'-CAGTCTGTGYTGACG CAGCCGCC-3'	SEQ ID NO:38
HuVλ1C	5'-CAGTCTGTCGTGACG CAGCCGCC-3'	SEQ ID NO:39
HuVλ2	5'-CARTCTGCCCTGACT CAGCCT-3'	SEQ ID NO:40
HuVλ3A	5'-TCCTATGWGCTGACT CAGCCACC-3'	SEQ ID NO:41
HuVλ3B	5'-TCTTCTGAGCTGACT CAGGACCC-3'	SEQ ID NO:42
HuVλ4	5'-CACGTTATACTGACT CAACCGCC-3'	SEQ ID NO:43
HuVλ5	5'-CAGGCTGTGCTGACT CAGCCGTC-3'	SEQ ID NO:44
HuVλ6	5'-AATTTTATGCTGACT CAGCCCCA-3'	SEQ ID NO:45
HuVλ7/8	5'-CAGRCTGTGGTGACY CAGGAGCC-3'	SEQ ID NO:46
HuVλ9	5'-CWGCCTGTGCTGACT CAGCCMCC-3'	SEQ ID NO:47

Table 6: Human kappa chain variable region primers (sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVκ1B	5'-GACATCCAGWTGACCC AGTCTCC-3'	SEQ ID NO:48
HuVκ2	5'-GATGTTGTGATGACT CAGTCTCC-3'	SEQ ID NO:49
HuVκ3	5'-GAAATTGTGWTGACR CAGTCTCC-3'	SEQ ID NO:50
HuVκ4	5'-GATATTGTGATGACC CACACTCC-3'	SEQ ID NO:51
HuVκ5	5'-GAAACGACACTCACG CAGTCTCC-3'	SEQ ID NO:52
HuVκ6	5'-GAAATTGTGCTGACTC AGTCTCC-3'	SEQ ID NO:53

Table 7: Human lambda chain J-region primers (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuJλ1	5'-ACCTAGGACGGTGACC TTGGTCCC-3'	SEQ ID NO:54
HuJλ2/3	5'-ACCTAGGACGGTCAG CTTGGTCCC-3'	SEQ ID NO:55
HuJλ4/5	5'-ACYTAAAACGGTGAG CTGGGTCCC-3'	SEQ ID NO:56

Table 8: Human lambda chain J-region primers (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
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HuJk1	5'-ACGTTTGATTTCAC CTTGGTCCC-3'	SEQ ID NO:57
HuJk2	5'-ACGTTTGATCTCCAG CTTGGTCCC-3'	SEQ ID NO:58
HuJk3	5'-ACGTTTGATATCCAC TTTGGTCCC-3'	SEQ ID NO:59
HuJk4	5'-ACGTTTGATCTCCAC CTTGGTCCC-3'	SEQ ID NO:60
HuJk5	5'-ACGTTTAATCTCCAG TCGTGTCCC-3'	SEQ ID NO:61

Table 9: Human kappa chain variable region primers extended with SalI restriction sites (sense), human kappa chain J-region primers extended with NotI restriction sites (anti-sense), human lambda chain variable region primers extended with SalI restriction sites (sense) and human lambda chain J-region primers extended with NotI restriction sites (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVk1B-SalI	5'-TGAGCACACAGGTCG ACGGACATCCAGWTGACC CAGTCTCC-3'	SEQ ID NO:62
HuVk2-SalI	5'-TGAGCACACAGGTCG ACGGATGTTGTGATGACT CAGTCTCC-3'	SEQ ID NO:63
HuVk3B-SalI	5'-TGAGCACACAGGTCG ACGGAAATTGTGWTGACR CAGTCTCC-3'	SEQ ID NO:64
HuVk4B-SalI	5'-TGAGCACACAGGTCG	SEQ ID NO:65

	ACGGATATTGTGATGACC CACACTCC-3'	
HuVk5-SalI	5'-TGAGCACACAGGTCGACG GAAACGACACTCACGCAGTCT CC-3'	SEQ ID NO:66
HuVk6-SalI	5'-TGAGCACACAGGTCG ACGGAAATTGTGCTGACT CAGTCTCC-3'	SEQ ID NO:67
HuJk1-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTGATTTCCAC CTTGGTCCC-3'	SEQ ID NO:68
HuJk2-NotI	5'-GAGTCATTCTCGACT TGCGGCCGCACGTTTGAT CTCCAGCTTGGTCCC-3'	SEQ ID NO:69
HuJk3-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTGATATCCAC TTTGGTCCC-3'	SEQ ID NO:70
HuJk4-NotI	5'-GAGTCATTCTCGACT TGCGGCCGCACGTTTGAT CTCCACCTTGGTCCC-3'	SEQ ID NO:71
HuJk5-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTAATCTCCAG TCGTGTCCC-3'	SEQ ID NO:72
HuVλ1A-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTGCTGACTCAGCCA CC-3'	SEQ ID NO:73
HuVλ1B-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTGYTGACGCAGCCG CC-3'	SEQ ID NO:74
HuVλ1C-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTCGTGACGCAGCCG	SEQ ID NO:75

	CC-3'	
HuVλ2-SalI	5'-TGAGCACACAGGTCGACG CARTCTGCCCTGACTCAGCCT- 3'	SEQ ID NO:76
HuVλ3A-SalI	5'-TGAGCACACAGGTCGACG TCCTATGWGCTGACTCAGCCA CC-3'	SEQ ID NO:77
HuVλ3B-SalI	5'-TGAGCACACAGGTCGACG TCTTCTGAGCTGACTCAGGAC CC-3'	SEQ ID NO:78
HuVλ4-SalI	5'-TGAGCACACAGGTCGACG CACGTTATACTGACTCAACCG CC-3'	SEQ ID NO:79
HuVλ5-SalI	5'-TGAGCACACAGGTCGACG CAGGCTGTGCTGACTCAGCCG TC-3'	SEQ ID NO:80
HuVλ6-SalI	5'-TGAGCACACAGGTCGACG AATTTTATGCTGACTCAGCCC CA-3'	SEQ ID NO:81
HuVλ7/8-SalI	5'-TGAGCACACAGGTCGACG CAGRCTGTGGTGACYCAGGAG CC-3'	SEQ ID NO:82
HuVλ9-SalI	5'-TGAGCACACAGGTCGACG CWGCCTGTGCTGACTCAGCCM CC-3'	SEQ ID NO:83
HuJλ1-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACCTAGGACGGTGAC CTTGGTCCC-3'	SEQ ID NO:84
HuJλ2/3-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACCTAGGACGGTCAG CTTGGTCCC-3'	SEQ ID NO:85

HuJλ4/5-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACYTAAAACGGTGAG CTGGGTCCC-3'	SEQ ID NO:86
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Table 10: Distribution of the different light chain products over the 10 fractions.

Light chain products	Number of alleles	Fraction number	alleles/fraction
Vk1B/Jk1-5	19	1 and 2	9.5
Vk2/Jk1-5	9	3	9
Vk3B/Jk1-5	7	4	7
Vk4B/Jk1-5	1	5	5
Vk5/Jk1-5	1		
Vk6/Jk1-5	3		
Vλ1A/J11-3	5	6	5
Vλ1B/J11-3			
Vλ1C/J11-3			
Vλ2/J11-3	5	7	5
Vλ3A/J11-3	9	8	9
Vλ3B/J11-3			
Vλ4/J11-3	3	9	5
Vλ5/J11-3	1		
Vλ6/J11-3	1		
Vλ7/8/J11-3	3	10	6
Vλ9/J11-3	3		

5

Table 11: Data of the single-chain Fvs capable of binding SARS-CoV.

Name scFv	SEQ ID NO of nucleo- tide sequence	SEQ ID NO of amino acid sequence	HCDR3	VH- germline	VL- germline
SC03-014	92	93	GISPFYFDY (SEQ ID NO:1)	V _H 3 DP29 (V _H 3-72)	V _k I DPK9 (02/012)
SC03-022	94	95	GSGISTPMDV (SEQ ID NO:2)	V _H 5-51 (DP-73)	V _k IV (B3- DPK24)

Table 12A: SARS-CoV neutralization experiment I.

	Antibody	CR03-014			CR03-022			cIgG1			Convalescent serum		
dilution		1	2	3	4	5	6	7	8	9	10	11	12
4 x	A	-	-	-	-	-	-	+	+	+	-	-	-
8 x	B	-	-	-	+	+	+	+	+	+	-	-	-
16 x	C	-	-	-	-	-	-	+	+	+	-	-	-
32 x	D	-	-	-	-	-	+	+	+	+	-	-	-
64 x	E	-	-	-	-	-	-	+	+	+	-	-	-
128 x	F	-	-	-	+	+	+	+	+	+	-	-	-
256 x	G	-	-	-	+	+	+	+	+	+	+	+	+
512 x	H	+	+	+	+	+	+	+	+	+	+	+	+

Table 12B: SARS-CoV neutralization experiment II.

[illegible]

Table 12C: SARS-CoV neutralization experiment III.

[illegible]

-: No CPE

+: CPE

- 5 Table 13: Concentrations of the monoclonal anti-SARS-CoV antibody CR03-014 giving complete protection against 100 TCID₅₀ of the different SARS-CoV isolates indicated in an *in vitro* neutralization assay.

SARS-CoV Strain*	Concentration of CR03-014 (µg/ml) resulting in 100% protection against 100 TCID ₅₀
36 (3)	12.5
39849 (3)	12.5
66 (2)	12.5
61567 (2)	12.5
61644 (1)	12.5
61644 (15)	12.5
GZ43 ¹	12.5
GZ50 ¹	12.5

10 * Between brackets the passage numbers of the respective strains is indicated. ¹ For strains GZ43 and GZ50 the passage history is unknown.

15 Table 14: SARS-CoV neutralization experiment of several mixtures of CR03-014 and CR03-022.

Mix	CR03-014 (625 µg/ml) %	CR03-022 (2.5 mg/ml) %	Wells protected at indicated dilution							Titer
			16x	32x	64x	128x	256x	512x	1024x	

A	0	100	6/6	6/6	5/6	3/6	3/6	0/6	0/6	64
B	10	90	6/6	6/6	6/6	6/6	6/6	2/6	0/6	256
C	50	50	6/6	6/6	6/6	6/6	6/6	6/6	4/6	>1024
D	90	10	ND	6/6	6/6	6/6	4/6	1/6	0/6	256
E	100	0	5/6	2/6	0/6	0/6	0/6	0/6	0/6	16

Table 15: List of SARS-CoV strains having a region 318-510 of the S protein not identical to the region 318-510 of the S protein of SARS-CoV Frankfurt 1 strain.

Mutation	Strain	Genbank
K344R	GZ02	AY390556
	GZ60	AY304491
	JMD	AY394988
	ZS-B	AY394996
	GZ43	AY304490
	HGZ8L1-A	AY394981
	ZS-A	AY394997
	ZS-C	AY395003
K344R	GD01	AY278489
F501Y		
K344R	GD03T0013	AY525636
F360S		
L472P		
D480G		
T487S		
S353F	Sin3408	AY559083
	Sin3765V	AY559084
	Sin845	AY559093
	Sin847	AY559095

	Sin849	AY559086
	Sin852	AY559082
	Sin3725V	AY559087
	Sin842	AY559081
	Sin846	AY559094
	Sin848	AY559085
	Sin850	AY559096
R426G N437D	Shanghai LY	AY322205S3
Y436H	GZ-C	AY394979
Y442S	Sino1-11	AY485277
N479S	BJ302 cl. 2	AY429073
	BJ302 cl. 4	AY429075
	BJ302 cl. 6	AY429077
	BJ302 cl. 3	AY429074
	BJ302 cl. 5	AY429076
	BJ302 cl. 8	AY429079

The amino acid substitutions compared to the Frankfurt 1 S protein are indicated in the left column. Strain and GenBank accession number are indicated in second and third column.

5

Table 16. Neutralization Index of E014 escape viruses

Escape virus	Log (TCID ₅₀ /ml) without mAb	Log (TCID ₅₀ /ml) with mAb	NI
E014-C06	7.45	7.53	0
E014-C07	7.07	6.85	0.22
E014-C08	7.83	7.1	0.73
E014-C09	7.53	6.85	0.68
E014-C10	6.77	7.1	0
Wt SARS-	7.23	3.93	3.3

CoV			
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Table 17. Neutralization index of putative E022 escape viruses

Virus	Log (TCID ₅₀ /ml) without mAb	Log (TCID ₅₀ /ml) with mAb	NI	Escape
Wt SARS-CoV	7.3	4	3.3	No
E22-2	7.3	2.5	4.8	No
E22-3	7.45	2.5	4.95	No
E22-5	7.3	2.7	4.6	No
E22-6	7.3	2.95	4.35	No
E22-9	8.1	3.7	4.4	No

5

Table 18. CR03-022 neutralizing titers for two representative escape viruses of CR03-014

Virus	Titer (µg/ml) ^a	Titer (µg/ml) ^b
Wild-type	35.46	50.00
E14-6	25.00	50.00
E14-7	31.50	50.00

^a 50% neutralizing titer of CR03-022 for each virus is indicated in µg/ml

10 ^b 100% neutralizing titer of CR03-022 for each virus is indicated in µg/ml

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REFERENCES

Boel E, Verlaan S, Poppelier MJ, Westerdaal NA, Van Strijp JA and Logtenberg T (2000), Functional human monoclonal
 5 antibodies of all isotypes constructed from phage display library-derived single-chain Fv antibody fragments. J. Immunol. Methods 239:153-166.

Burton DR and Barbas CF (1994), Human antibodies from combinatorial libraries. Adv. Immunol. 57:191-280.

10 Chou, TC and P Talalay (1984), Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 22:27-55.

De Kruif J, Terstappen L, Boel E and Logtenberg T (1995a), Rapid selection of cell subpopulation-specific human
 15 monoclonal antibodies from a synthetic phage antibody library. Proc. Natl. Acad. Sci. USA 92:3938.

De Kruif J, Boel E and Logtenberg T (1995b), Selection and application of human single-chain Fv antibody fragments from a semi-synthetic phage antibody display library with
 20 designed CDR3 regions. J. Mol. Biol. 248:97-105.

Emini EA, Nara PL, Schleif WA, Lewis JA, Davide JP, Lee DR, Kessler J, Conley S, Matsushita S, Putney SDJ, Gerety RJ and Eichberg JW (1990), Antibody-mediated *in vitro*
 neutralization of human immunodeficiency virus type 1
 25 abolishes infectivity for chimpanzees. J. Virol. 64:3674-3678.

Fouchier RA, Kuiken T, Schutten M, van Amerongen G, van Doornum GJ, van den Hoogen BG, Peiris M, Lim W, Stohr K, Osterhaus AD (2003), Koch's postulates fulfilled for SARS virus. Nature 243:240.

30 Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, and Poon LL (2003),

Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302:276-278.

Havenga MJ, Lemckert AA, Grimbergen JM, Vogels R, Huisman
5 LG, Valerio D, Bout A and Quax PH (2001), Improved adenovirus
vectors for infection of cardiovascular tissues. *J. Virol.*
75:3335-3342.

Holmes KV (2003), SARS coronavirus: a new challenge for
prevention and therapy. *J. Clin. Invest.* 111:1605-1609.

10 Huls G, Heijnen IJ, Cuomo E, van der Linden J, Boel E,
van de Winkel J and Logtenberg T (1999), Antitumor immune
effector mechanisms recruited by phage display-derived fully
human IgG1 and IgA1 monoclonal antibodies. *Cancer Res.*
59:5778-5784.

15 Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T,
Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell
SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD,
Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc
JW, Bellini WJ, Anderson LJ (2003), A novel coronavirus
20 associated with severe acute respiratory syndrome. *N. Eng. J.*
Med. 348:1953-1966.

Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van
Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G,
Lim W, Ling AE, Chan PK, Tam JS, Zambon MC, Gopal R, Drosten
25 C, van der Werf S, Escriou N, Manuguerra JC, Stohr K, Peiris
JS and Osterhaus AD (2003), Newly discovered coronavirus as
the primary cause of severe acute respiratory syndrome. *Lancet*
362:263-270.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA,
30 Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H
and Farzan M (2003), Angiotensin-converting enzyme 2 is a

functional receptor for the SARS coronavirus. *Nature* 2003
426:450-454.

Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A,
Butterfield YS, Khattra J, Asano JK, Barber SA, Chan SY,
5 Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach
SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS,
Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM,
Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K,
Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R,
10 Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A,
Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S,
Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M,
Skowronski DM, Upton C, Roper RL (2003), The genome sequence
of the SARS-associated coronavirus. *Science* 300, 1399-1404.

15 Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY,
Chu CM, Hui PK, Mak KL, Lim W, Yan KW, Chan KH, Tsang NC,
Guan Y, Yuen KY, Peiris JS (2003), Lung pathology of fatal
severe acute respiratory syndrome. *Lancet* 361:1773-1778.

Poon LLM, Leung CSW, Yuen KH, Guan Y, and Peiris JSM
20 (2005), Recurrent mutations associated with isolation and
passage of SARS Coronavirus in cells from non-human primates.
J. Med. Virol. 76:435-440.

Rickerts V, Wolf T, Rottmann C, Preiser W, Drosten C,
Jakobi V, Leong HN, Brodt HR (2003), Klinik und Behandlung des
25 schweren akuten respiratorischen Syndroms. *Dtsch. Med.*
Wochenschrift 128:1109-1114.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R,
Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S,
Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman
30 DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A,
Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen
M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch

MA, Anderson LJ, Bellini WJ. (2003), Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 300:1394-1399.

5 Sloodstra JW, Puijk WC, Ligtvoet GJ, Langeveld JP, Meloen RH (1996), Structural aspects of antibody-antigen interaction revealed through small random peptide libraries. *Mol. Divers.* 1:87-96.

10 Ter Meulen J, Bakker ABH, van den Brink EN, Weverling GJ, Martina BEE, Haagmans BL, Kuiken T, de Kruif J, Preiser W, Spaan W, Gelderblom HR, Goudsmit J, Osterhaus ADME (2004), Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. *The Lancet* 363:2139-2141.

SEQUENCE LISTING

<110> Crucell Holland B.V.
 <120> Compositions against SARS-coronavirus and uses thereof
 <130> PAT 63777W-1
 <140> 2,582,057
 <141> 2005-11-10
 <150> EP 04105684.7
 <151> 2004-11-11
 <150> US 60/627,773
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Gly Ile Ser Pro Phe Tyr Phe Asp Tyr
 1 5

<210> 2
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> HCDR3 of SC03-022

<400> 2

Gly Ser Gly Ile Ser Thr Pro Met Asp Val
 1 5 10

<210> 3
 <211> 366
 <212> DNA
 <213> Artificial sequence

2

<220>
 <221> CDS
 <222> (1)..(366)
 <223> Variable heavy chain of SC03-014

<400> 3
 atg gcc gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct 48
 Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
 1 5 10 15
 gga ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttc agt 96
 Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 20 25 30
 gac cac tac atg gac tgg gtc cgc cag gct cca ggg aag ggg ctg gag 144
 Asp His Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 tgg gtt ggc cgt act aga aac aaa gct aac agt tac acc aca gaa tac 192
 Trp Val Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr
 50 55 60
 gcc gcg tct gtg aaa ggc aga ttc acc atc tca aga gat gat tca aag 240
 Ala Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys
 65 70 75 80
 aac tca ctg tat ctg caa atg aac agc ctg aaa acc gag gac acg gcc 288
 Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala
 85 90 95
 gtg tat tac tgt gca agg ggg att tcg ccg ttt tac ttt gac tac tgg 336
 Val Tyr Tyr Cys Ala Arg Gly Ile Ser Pro Phe Tyr Phe Asp Tyr Trp
 100 105 110
 ggc caa ggt acc ctg gtc acc gtc tcg agt 366
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 4
 <211> 122
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Variable heavy chain of SC03-014

<400> 4
 Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
 1 5 10 15
 Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 20 25 30

<400>	5															
atg gcc cag atg cag ctg gtg caa tct gga aca gag gtg aaa aag ccg																48
Met Ala Gln Met Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro																
1	5				10					15						
ggg gag tct ctg aag atc tcc tgt aag ggt tct gga tac ggc ttt atc																96
Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Gly Phe Ile																
	20				25					30						
acc tac tgg atc ggc tgg gtg cgc cag atg ccc ggg aaa ggc ctg gag																144
Thr Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu																
	35				40					45						
tgg atg ggg atc atc tat cct ggt gac tct gaa acc aga tac agc ccg																192
Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro																
	50				55					60						
tcc ttc caa ggc cag gtc acc atc tca gcc gac aag tcc atc aac acc																240
Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Asn Thr																
65	70				75					80						
gcc tac ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc ata tat																288
Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile Tyr																
	85				90					95						

4

tac	tgt	gcg	ggg	ggt	tcg	ggg	att	tct	acc	cct	atg	gac	gtc	tgg	ggc	336
Tyr	Cys	Ala	Gly	Gly	Ser	Gly	Ile	Ser	Thr	Pro	Met	Asp	Val	Trp	Gly	
			100					105					110			

caa	ggg	acc	acg	gtc	acc	gtc	tcg	agc								363
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
		115					120									

<210> 6
 <211> 121
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Variable heavy chain of SC03-022

<400> 6

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

Gly	Glu	Ser	Leu	Lys	Ile	Ser	Cys	Lys	Gly	Ser	Gly	Tyr	Gly	Phe	Ile
			20					25					30		

Thr	Tyr	Trp	Ile	Gly	Trp	Val	Arg	Gln	Met	Pro	Gly	Lys	Gly	Leu	Glu
		35					40					45			

Trp	Met	Gly	Ile	Ile	Tyr	Pro	Gly	Asp	Ser	Glu	Thr	Arg	Tyr	Ser	Pro
	50					55					60				

Ser	Phe	Gln	Gly	Gln	Val	Thr	Ile	Ser	Ala	Asp	Lys	Ser	Ile	Asn	Thr
65					70					75				80	

Ala	Tyr	Leu	Gln	Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala	Ile	Tyr
				85					90					95	

Tyr	Cys	Ala	Gly	Gly	Ser	Gly	Ile	Ser	Thr	Pro	Met	Asp	Val	Trp	Gly
			100					105					110		

Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser							
		115					120								

<210> 7
 <211> 318
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(318)
 <223> Variable light chain of SC03-014

5

<400> 7
gag ctc acc cag tct cca tcc tcc ctg tct gca tct gta gga gac aga 48
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1 5 10 15
gtc acc atc act tgc cgg gca agt cag agc att agc agc tac tta aat 96
Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
20 25 30
tgg tat cag cag aaa cca ggg aaa gcc cct aag ctc ctg atc tat gct 144
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
35 40 45
gca tcc agt ttg caa agt ggg gtc cca tca agg ttc agt ggc agt gga 192
Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
50 55 60
tct ggg aca gat ttc act ctc acc atc agc agt ctg caa cct gaa gat 240
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65 70 75 80
ttt gca act tac tac tgt caa cag agt tac agt acc cct cca acg ttc 288
Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Pro Thr Phe
85 90 95
ggc caa ggg acc aag gtg gag atc aaa cgt 318
Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 8
<211> 106
<212> PRT
<213> Artificial sequence

<220>
<223> Variable light chain of SC03-014

<400> 8
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1 5 10 15
Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
20 25 30
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
35 40 45
Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
50 55 60
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp

6

65		70		75		80									
Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Pro	Thr	Phe
				85					90					95	
Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg						
			100					105							

<210> 9
 <211> 348
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(348)
 <223> Variable light chain of SC03-022

<400> 9

gac	atc	cag	ttg	acc	cag	tct	cca	gac	tcc	ctg	gct	gtg	tct	ctg	ggc	48
Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly	
1				5					10					15		
gag	agg	gcc	acc	atc	aac	tgc	aag	tcc	agc	cag	agt	gtt	tta	tac	agc	96
Glu	Arg	Ala	Thr	Ile	Asn	Cys	Lys	Ser	Ser	Gln	Ser	Val	Leu	Tyr	Ser	
			20					25					30			
tcc	atc	aat	aag	aac	tac	tta	gct	tgg	tac	cag	cag	aaa	cca	gga	cag	144
Ser	Ile	Asn	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	
			35				40					45				
cct	cct	aag	ctg	ctc	att	tac	tgg	gca	tct	acc	cgg	gaa	tcc	ggg	gtc	192
Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Thr	Arg	Glu	Ser	Gly	Val	
			50			55					60					
cct	gac	cga	ttc	agt	ggc	agc	ggg	tct	ggg	aca	gat	ttc	act	ctc	acc	240
Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	
65					70				75					80		
atc	agc	agc	ctg	cag	gct	gaa	gat	gtg	gca	gtt	tat	tac	tgt	cag	caa	288
Ile	Ser	Ser	Leu	Gln	Ala	Glu	Asp	Val	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	
				85					90					95		
tat	tat	agt	act	ccg	tac	act	ttt	ggc	cag	ggg	acc	aag	gtg	gaa	atc	336
Tyr	Tyr	Ser	Thr	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	
			100					105					110			
aaa	cgt	gcg	gcc													348
Lys	Arg	Ala	Ala													
			115													

<210> 10

7

<211> 116
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Variable light chain of SC03-022

<400> 10

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

<210> 11
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH1B/7A

<400> 11
 cagrtgcagc tgggtgcartc tgg

23

<210> 12
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH1C

<400> 12
 saggtccagc tggtrcagtc tgg 23

<210> 13
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH2B

<400> 13
 saggtgcagc tgggtggagtc tgg 23

<210> 14
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH3B

<400> 14
 saggtgcagc tgggtggagtc tgg 23

<210> 15
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH3C

<400> 15
 gaggtgcagc tgggtggagwc ygg 23

<210> 16
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH4B

<400> 16
 caggtgcagc tacagcagtg ggg 23

<210> 17
 <211> 23

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH4C

<400> 17
 cagstgcagc tgcaggagtc sgg 23

<210> 18
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH5B

<400> 18
 gargtgcagc tgggtgcagtc tgg 23

<210> 19
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVH6A

<400> 19
 caggtacagc tgcagcagtc agg 23

<210> 20
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJH1/2

<400> 20
 tgaggagacg gtgaccaggg tgcc 24

<210> 21
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJH3

10

<400> 21
tgaagagacg gtgaccattg tccc 24

<210> 22
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJH4/5

<400> 22
tgaggagacg gtgaccaggg ttcc 24

<210> 23
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJH6

<400> 23
tgaggagacg gtgaccgtgg tccc 24

<210> 24
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVH1B/7A-NcoI

<400> 24
gtcctcgcaa ctgcggccca gccggccatg gccagrtgc agctgggtgca rtctgg 56

<210> 25
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVH1C-NcoI

<400> 25
gtcctcgcaa ctgcggccca gccggccatg gccsaggtcc agctggtrca gtctgg 56

<210> 26
<211> 56

11

<212> DNA
 <213> Artificial sequence

<220>

<223> Primer HuVH2B-NcoI

<400> 26

gtcctcgcaa ctgcggccca gccggccatg gcccagrtca ccttgaagga gtctgg 56

<210> 27

<211> 56

<212> DNA

<213> Artificial sequence

<220>

<223> Primer HuVH3B-NcoI

<400> 27

gtcctcgcaa ctgcggccca gccggccatg gccsaggtgc agctggtgga gtctgg 56

<210> 28

<211> 56

<212> DNA

<213> Artificial sequence

<220>

<223> Primer HuVH3C-NcoI

<400> 28

gtcctcgcaa ctgcggccca gccggccatg gccgaggtgc agctggtgga gwcyyg 56

<210> 29

<211> 56

<212> DNA

<213> Artificial sequence

<220>

<223> Primer HuVH4B-NcoI

<400> 29

gtcctcgcaa ctgcggccca gccggccatg gcccaggtgc agctacagca gtgggg 56

<210> 30

<211> 56

<212> DNA

<213> Artificial sequence

<220>

<223> Primer HuVH4C-NcoI

<400> 30
gtcctcgcaa ctgcggccca gccggccatg gcccagstgc agctgcagga gtcsgg 56

<210> 31
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVH5B-NcoI

<400> 31
gtcctcgcaa ctgcggccca gccggccatg gccgargtgc agctgggtgca gtctgg 56

<210> 32
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVH6A-NcoI

<400> 32
gtcctcgcaa ctgcggccca gccggccatg gcccaggtag agctgcagca gtcagg 56

<210> 33
<211> 36
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJH1/2-XhoI

<400> 33
gagtcattct cgactcgaga cggtgaccag ggtgcc 36

<210> 34
<211> 36
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJH3-XhoI

<400> 34
gagtcattct cgactcgaga cggtgaccat tgtccc 36

<210> 35
<211> 36

13

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJH4/5-XhoI

<400> 35
 gagtcattct cgactcgaga cggtgaccag ggttcc 36

<210> 36
 <211> 36
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJH6-XhoI

<400> 36
 gagtcattct cgactcgaga cggtgaccgt ggtccc 36

<210> 37
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda1A

<400> 37
 cagtctgtgc tgactcagcc acc 23

<210> 38
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda1B

<400> 38
 cagtctgtgy tgacgcagcc gcc 23

<210> 39
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda1C

14

<400> 39
cagtctgtcg tgacgcagcc gcc 23

<210> 40
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVlambda2

<400> 40
cartctgccc tgactcagcc t 21

<210> 41
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVlambda3A

<400> 41
tcctatgwgcc tgactcagcc acc 23

<210> 42
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVlambda3B

<400> 42
tcttctgagc tgactcagga ccc 23

<210> 43
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVlambda4

<400> 43
cacgttatac tgactcaacc gcc 23

<210> 44
<211> 23

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda5

<400> 44
 caggctgtgc tgactcagcc gtc

23

<210> 45
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda6

<400> 45
 aattttatgc tgactcagcc cca

23

<210> 46
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda7/8

<400> 46
 cagrcctgtgg tgacycagga gcc

23

<210> 47
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVlambda9

<400> 47
 cwgccctgtgc tgactcagcc mcc

23

<210> 48
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa1B

<400> 48
gacatccagw tgacccagtc tcc 23

<210> 49
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVkappa2

<400> 49
gatgttgtga tgactcagtc tcc 23

<210> 50
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVkappa3

<400> 50
gaaattgtgw tgacrcagtc tcc 23

<210> 51
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVkappa4

<400> 51
gatattgtga tgaccacac tcc 23

<210> 52
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVkappa5

<400> 52
gaaacgacac tcacgcagtc tcc 23

<210> 53
<211> 23

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa6

<400> 53
 gaaattgtgc tgactcagtc tcc

23

<210> 54
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJlambda1

<400> 54
 acctaggacg gtgaccttgg tccc

24

<210> 55
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJlambda2/3

<400> 55
 acctaggacg gtcagcttgg tccc

24

<210> 56
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJlambda4/5

<400> 56
 acytaaaacg gtgagctggg tccc

24

<210> 57
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJkappa1

<400> 57
acgttttgatt tccaccttgg tccc 24

<210> 58
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa2

<400> 58
acgtttgatc tccagcttgg tccc 24

<210> 59
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa3

<400> 59
acgtttgata tccactttgg tccc 24

<210> 60
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa4

<400> 60
acgtttgatc tccaccttgg tccc 24

<210> 61
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa5

<400> 61
acgtttaatc tccagtcgtg tccc 24

<210> 62
<211> 41

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa1B-SalI

<400> 62
 tgagcacaca ggtcgacgga catccagwtg acccagtctc c 41

<210> 63
 <211> 41
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa2-SalI

<400> 63
 tgagcacaca ggtcgacgga tgttgtgatg actcagtctc c 41

<210> 64
 <211> 41
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa3B-SalI

<400> 64
 tgagcacaca ggtcgacgga aattgtgwtg acrcagtctc c 41

<210> 65
 <211> 41
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa4B-SalI

<400> 65
 tgagcacaca ggtcgacgga tattgtgatg acccacactc c 41

<210> 66
 <211> 41
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuVkappa5-SalI

20

<400> 66
tgagcacaca ggtcgacgga aacgacactc acgcagtctc c 41

<210> 67
<211> 41
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuVkappa6-SalI

<400> 67
tgagcacaca ggtcgacgga aattgtgctg actcagtctc c 41

<210> 68
<211> 48
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa1-NotI

<400> 68
gagtcattct cgacttgcgg ccgcacgttt gatttccacc ttggtccc 48

<210> 69
<211> 48
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa2-NotI

<400> 69
gagtcattct cgacttgcgg ccgcacgttt gatctccagc ttggtccc 48

<210> 70
<211> 48
<212> DNA
<213> Artificial sequence

<220>
<223> Primer HuJkappa3-NotI

<400> 70
gagtcattct cgacttgcgg ccgcacgttt gatatccact ttggtccc 48

<210> 71
<211> 48

21

<212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJkappa4-NotI

<400> 71
 gagtcattct cgacttgcg cgcacgttt gatctccacc ttggtccc 48

<210> 72
 <211> 48
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer HuJ?kappa5-NotI

<400> 72
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22

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<400> 76
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<210> 80
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<400> 82
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<400> 83
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24

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<210> 86
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<400> 86
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<210> 87
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<211> 24

28

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<220>
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<400> 89
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<210> 90
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<220>
 <223> Primer HuClambda2

<400> 90
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<210> 91
 <211> 23
 <212> DNA
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<220>
 <223> Primer HuClambda7

<400> 91
 agagcattct gcaggggccca ctg 23

<210> 92
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 <223> SC03-014

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cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt 96
 Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 20 25 30

agc gac tac ttg atg aac tgg gtc cgc cag gcg ccc ggg aag ggg ctg 144

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Glu	Trp	Val	Gly	Arg	Ile	Arg	Ser	Lys	Ala	Asn	Ser	Tyr	Ala	Thr	Ala	
	50					55					60					
tat	gct	gcg	tcg	gtg	aaa	ggc	agg	ttc	acc	atc	tcc	aga	gat	gat	tca	240
Tyr	Ala	Ala	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	
65					70					75					80	
aag	aac	acg	gcg	tat	ctg	caa	atg	aac	agc	ctg	aaa	acc	gag	gac	acg	288
Lys	Asn	Thr	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	
				85					90					95		
gcc	gtg	tat	tac	tgt	gct	aaa	gac	ggc	agc	cgg	ttc	ccc	gcc	cgc	ttc	336
Ala	Val	Tyr	Tyr	Cys	Ala	Lys	Asp	Gly	Ser	Arg	Phe	Pro	Ala	Arg	Phe	
			100					105					110			
gat	tac	tgg	ggc	cag	ggc	acc	ctg	gtg	acc	gtg	ctc	gag	ggg	acc	gga	384
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Leu	Glu	Gly	Thr	Gly	
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Gly	Ser	Gly	Gly	Thr	Gly	Ser	Gly	Thr	Gly	Thr	Ser	Glu	Leu	Thr	Gln	
	130					135					140					
tct	cca	tcc	tcc	ctg	tct	gca	tct	gta	gga	gac	aga	gtc	acc	atc	act	480
Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	
145					150					155					160	
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Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	
				165					170					175		
aaa	cca	ggg	aaa	gcc	cct	aag	ctc	ctg	atc	tat	gct	gca	tcc	agt	ttg	576
Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Ser	Leu	
			180					185					190			
caa	agt	ggg	gtc	cca	tca	agg	ttc	agt	ggc	agt	gga	tct	ggg	aca	gat	624
Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	
		195					200					205				
ttc	act	ctc	acc	atc	agc	agt	ctg	caa	cct	gaa	gat	ttt	gca	act	tac	672
Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	
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Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	
225					230					235					240	
aag	gtg	gag	atc	aaa	cgt	gcg	gcc	gca								747
Lys	Val	Glu	Ile	Lys	Arg	Ala	Ala	Ala								
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<220>
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<400> 93

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Ser	Asp	Tyr	Leu	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	35	40	45	
Glu	Trp	Val	Gly	Arg	Ile	Arg	Ser	Lys	Ala	Asn	Ser	Tyr	Ala	Thr	Ala	50	55	60	
Tyr	Ala	Ala	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	65	70	75	80
Lys	Asn	Thr	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	85	90	95	
Ala	Val	Tyr	Tyr	Cys	Ala	Lys	Asp	Gly	Ser	Arg	Phe	Pro	Ala	Arg	Phe	100	105	110	
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Leu	Glu	Gly	Thr	Gly	115	120	125	
Gly	Ser	Gly	Gly	Thr	Gly	Ser	Gly	Thr	Gly	Thr	Ser	Glu	Leu	Thr	Gln	130	135	140	
Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	145	150	155	160
Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	165	170	175	
Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Ser	Leu	180	185	190	
Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	195	200	205	
Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	210	215	220	

31

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Lys Val Glu Ile Lys Arg Ala Ala Ala
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 <223> SC03-022

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 Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Gly Phe
 20 25 30
 atc acc tac tgg atc ggc tgg gtg cgc cag atg ccc ggg aaa ggc ctg 144
 Ile Thr Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu
 35 40 45
 gag tgg atg ggg atc atc tat cct ggt gac tct gaa acc aga tac agc 192
 Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser
 50 55 60
 ccg tcc ttc caa ggc cag gtc acc atc tca gcc gac aag tcc atc aac 240
 Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Asn
 65 70 75 80
 acc gcc tac ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc ata 288
 Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile
 85 90 95
 tat tac tgt gcg ggg ggt tcg ggg att tct acc cct atg gac gtc tgg 336
 Tyr Tyr Cys Ala Gly Gly Ser Gly Ile Ser Thr Pro Met Asp Val Trp
 100 105 110
 ggc caa ggg acc acg gtc acc gtc tcg agc ggt acg ggc ggt tca ggc 384
 Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Thr Gly Gly Ser Gly
 115 120 125
 gga acc ggc agc ggc act ggc ggg tcg acg gac atc cag ttg acc cag 432
 Gly Thr Gly Ser Gly Thr Gly Gly Ser Thr Asp Ile Gln Leu Thr Gln
 130 135 140

32

tct cca gac tcc ctg gct gtg tct ctg ggc gag agg gcc acc atc aac	480
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Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Ile Asn Lys Asn Tyr	
165 170 175	
tta gct tgg tac cag cag aaa cca gga cag cct cct aag ctg ctc att	576
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile	
180 185 190	
tac tgg gca tct acc cgg gaa tcc ggg gtc cct gac cga ttc agt ggc	624
Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly	
195 200 205	
agc ggg tct ggg aca gat ttc act ctc acc atc agc agc ctg cag gct	672
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala	
210 215 220	
gaa gat gtg gca gtt tat tac tgt cag caa tat tat agt act ccg tac	720
Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Tyr	
225 230 235 240	
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Ala Ala	
245 250	

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<220>
 <223> SC03-022

<400> 95

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35 40 45	
Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser	
50 55 60	
Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Asn	
65 70 75 80	

33

Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile
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 Tyr Tyr Cys Ala Gly Gly Ser Gly Ile Ser Thr Pro Met Asp Val Trp
 100 105 110
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tgaagtttta	aatcaatcta	aagtatatat	gagtaaactt	ggtctgacag	ttaccaatgc	5280
ttaatcagtg	aggcacctat	ctcagcgatc	tgtctatttc	gttcatccat	agttgcctga	5340
ctccccgtcg	tgtagataac	tacgatacgg	gagggcttac	catctggccc	cagtgcctgca	5400
atgataccgc	gagacccacg	ctcaccggct	ccagatttat	cagcaataaa	ccagccagcc	5460
ggaagggccg	agcgcagaag	tggtcctgca	actttatccg	cctccatcca	gtctattaat	5520
tgttgccggg	aagctagagt	aagtagttcg	ccagttaata	gtttgcgcaa	cgttggtgcc	5580
attgctacag	gcacgtgggt	gtcacgctcg	tcgtttggta	tggcttcatt	cagctccgggt	5640
tcccaacgat	caaggcgagt	tacatgatcc	cccatgttgt	gcaaaaaagc	ggttagctcc	5700
ttcggtcctc	cgatcgttgt	cagaagtaag	ttggccgcag	tgttatcact	catggttatg	5760
gcagcactgc	ataattctct	tactgtcatg	ccatccgtaa	gatgcttttc	tgtgactgggt	5820
gagtactcaa	ccaagtcatt	ctgagaatag	tgtatgcggc	gaccgagttg	ctcttgcccc	5880
gcgtcaatac	gggataatac	cgcgccacat	agcagaactt	taaaagtgct	catcattgga	5940
aaacgttctt	cggggcgaaa	actctcaagg	atcttaccgc	tgttgagatc	cagttcgatg	6000
taaccctctc	gtgcacccaa	ctgatcttca	gcacctttta	ctttcaccag	cgtttctggg	6060
tgagcaaaaa	caggaaggca	aatgcccga	aaaaagggaa	taagggcgac	acggaaatgt	6120
tgaatactca	tactcttctc	ttttcaatat	tattgaagca	tttatcaggg	ttattgtctc	6180
atgagcggat	acatatttga	atgtatttag	aaaaataaac	aataggggt	tccgcgcaca	6240

tttccccgaa aagtgccacc tgacgtc

6267

<210> 98
<211> 54
<212> DNA
<213> Artificial sequence

<220>
<223> Oligonucleotide 5K-I

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54

<210> 99
<211> 42
<212> DNA
<213> Artificial sequence

<220>
<223> Oligonucleotide sy3K-C

<400> 99
gctggggggcg gccacgggtcc gtttgatctc caccttggtc cc

42

<210> 100
<211> 49
<212> DNA
<213> Artificial sequence

<220>
<223> Oligonucleotide 5K-J

<400> 100
acctgtctcg agttttccat ggctgacatc gtgatgaccc agtctccag

49

<210> 101
<211> 42
<212> DNA
<213> Artificial sequence

<220>
<223> Oligonucleotide sy3K-F

<400> 101
gctggggggcg gccacgggtcc gcttgatctc caccttggtc cc

42

<210> 102
<211> 46

43

<212> DNA
 <213> Artificial sequence

<220>
 <223> Oligonucleotide 5H-B

<400> 102
 acctgtcttg aattctccat ggccgaggtg cagctggtgg agtctg

46

<210> 103
 <211> 47
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Oligonucleotide sy3H-A

<400> 103
 gcccttggtg ctagcgctgg agacgggtcac cagggtgccc tggcccc

47

<210> 104
 <211> 47
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Oligonucleotide 5H-H

<400> 104
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47

<210> 105
 <211> 47
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Oligonucleotide sy3H-C

<400> 105
 gcccttggtg ctagcgctgg agacgggtcac ggtggtgccc tggcccc

47

<210> 106
 <211> 1350
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(1350)

44

<223> IgG heavy chain of CR03-014

<400> 106

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Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
1				5					10					15		
tcc	ctg	aga	ctc	tcc	tgt	gca	gcc	tct	gga	ttc	acc	ttc	agt	gac	cac	96
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asp	His	
			20					25					30			
tac	atg	gac	tgg	gtc	cgc	cag	gct	cca	ggg	aag	ggg	ctg	gag	tgg	gtt	144
Tyr	Met	Asp	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
		35					40					45				
ggc	cgt	act	aga	aac	aaa	gct	aac	agt	tac	acc	aca	gaa	tac	gcc	gcg	192
Gly	Arg	Thr	Arg	Asn	Lys	Ala	Asn	Ser	Tyr	Thr	Thr	Glu	Tyr	Ala	Ala	
	50					55					60					
tct	gtg	aaa	ggc	aga	ttc	acc	atc	tca	aga	gat	gat	tca	aag	aac	tca	240
Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Ser	
65					70					75					80	
ctg	tat	ctg	caa	atg	aac	agc	ctg	aaa	acc	gag	gac	acg	gcc	gtg	tat	288
Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr	
				85					90					95		
tac	tgt	gca	agg	ggg	att	tcg	ccg	ttt	tac	ttt	gac	tac	tgg	ggc	cag	336
Tyr	Cys	Ala	Arg	Gly	Ile	Ser	Pro	Phe	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	
			100					105					110			
ggc	acc	ctg	gtg	acc	gtc	tcc	agc	gct	agc	acc	aag	ggc	ccc	agc	gtg	384
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
		115					120					125				
ttc	ccc	ctg	gcc	ccc	agc	agc	aag	agc	acc	agc	ggc	ggc	aca	gcc	gcc	432
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	
	130					135					140					
ctg	ggc	tgc	ctg	gtg	aag	gac	tac	ttc	ccc	gag	ccc	gtg	acc	gtg	agc	480
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
145					150					155					160	
tgg	aac	agc	ggc	gcc	ttg	acc	agc	ggc	gtg	cac	acc	ttc	ccc	gcc	gtg	528
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
				165					170					175		
ctg	cag	agc	agc	ggc	ctg	tac	agc	ctg	agc	agc	gtg	gtg	acc	gtg	ccc	576
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
			180					185					190			
agc	agc	agc	ctg	ggc	acc	cag	acc	tac	atc	tgc	aac	gtg	aac	cac	aag	624
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	
			195				200					205				

45

ccc agc aac acc aag gtg gac aaa cgc gtg gag ccc aag agc tgc gac Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp 210 215 220	672
aag acc cac acc tgc ccc ccc tgc cct gcc ccc gag ctg ctg ggc gga Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly 225 230 235 240	720
ccc tcc gtg ttc ctg ttc ccc ccc aag ccc aag gac acc ctc atg atc Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile 245 250 255	768
agc cgg acc ccc gag gtg acc tgc gtg gtg gtg gac gtg agc cac gag Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu 260 265 270	816
gac ccc gag gtg aag ttc aac tgg tac gtg gac ggc gtg gag gtg cac Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His 275 280 285	864
aac gcc aag acc aag ccc cgg gag gag cag tac aac agc acc tac cgg Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg 290 295 300	912
gtg gtg agc gtg ctc acc gtg ctg cac cag gac tgg ctg aac ggc aag Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys 305 310 315 320	960
gag tac aag tgc aag gtg agc aac aag gcc ctg cct gcc ccc atc gag Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu 325 330 335	1008
aag acc atc agc aag gcc aag ggc cag ccc cgg gag ccc cag gtg tac Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr 340 345 350	1056
acc ctg ccc ccc agc cgg gag gag atg acc aag aac cag gtg tcc ctc Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu 355 360 365	1104
acc tgt ctg gtg aag ggc ttc tac ccc agc gac atc gcc gtg gag tgg Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp 370 375 380	1152
gag agc aac ggc cag ccc gag aac aac tac aag acc acc ccc cct gtg Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val 385 390 395 400	1200
ctg gac agc gac ggc agc ttc ttc ctg tac agc aag ctc acc gtg gac Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp 405 410 415	1248
aag agc cgg tgg cag cag ggc aac gtg ttc agc tgc agc gtg atg cac	1296

46

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

gag gcc ctg cac aac cac tac acc cag aag agc ctg agc ctg agc ccc 1344
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 435 440 445

ggc aag 1350
 Gly Lys
 450

<210> 107
 <211> 450
 <212> PRT
 <213> Artificial sequence

<220>
 <223> IgG heavy chain of CR03-014

<400> 107

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
 20 25 30

Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
 50 55 60

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

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

Tyr Cys Ala Arg Gly Ile Ser Pro Phe Tyr Phe Asp Tyr Trp Gly Gln
 100 105 110

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

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
 130 135 140

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

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

[illegible]

48

<210> 108
 <211> 1347
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(1347)
 <223> IgG heavy chain of CR03-022

<400> 108
 gag gtg cag ctg gtg cag tct gga aca gag gtg aaa aag ccg ggg gag 48
 Glu Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Glu
 1 5 10 15
 tct ctg aag atc tcc tgt aag ggt tct gga tac ggc ttt atc acc tac 96
 Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Gly Phe Ile Thr Tyr
 20 25 30
 tgg atc ggc tgg gtg cgc cag atg ccc ggg aaa ggc ctg gag tgg atg 144
 Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 ggg atc atc tat cct ggt gac tct gaa acc aga tac agc ccg tcc ttc 192
 Gly Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro Ser Phe
 50 55 60
 caa ggc cag gtc acc atc tca gcc gac aag tcc atc aac acc gcc tac 240
 Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Asn Thr Ala Tyr
 65 70 75 80
 ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc ata tat tac tgt 288
 Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 gcg ggg ggt tcg ggg att tct acc cct atg gac gtc tgg ggc cag ggc 336
 Ala Gly Gly Ser Gly Ile Ser Thr Pro Met Asp Val Trp Gly Gln Gly
 100 105 110
 acc acc gtg acc gtc tcc agc gct agc acc aag ggc ccc agc gtg ttc 384
 Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 ccc ctg gcc ccc agc agc aag agc acc agc ggc ggc aca gcc gcc ctg 432
 Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 130 135 140
 ggc tgc ctg gtg aag gac tac ttc ccc gag ccc gtg acc gtg agc tgg 480
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160
 aac agc ggc gcc ttg acc agc ggc gtg cac acc ttc ccc gcc gtg ctg 528

49

Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	
				165					170					175		
cag	agc	agc	ggc	ctg	tac	agc	ctg	agc	agc	gtg	gtg	acc	gtg	ccc	agc	576
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	
			180				185						190			
agc	agc	ctg	ggc	acc	cag	acc	tac	atc	tgc	aac	gtg	aac	cac	aag	ccc	624
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
		195					200					205				
agc	aac	acc	aag	gtg	gac	aaa	cgc	gtg	gag	ccc	aag	agc	tgc	gac	aag	672
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
	210					215					220					
acc	cac	acc	tgc	ccc	ccc	tgc	cct	gcc	ccc	gag	ctg	ctg	ggc	gga	ccc	720
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
225					230					235					240	
tcc	gtg	ttc	ctg	ttc	ccc	ccc	aag	ccc	aag	gac	acc	ctc	atg	atc	agc	768
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
				245					250					255		
cgg	acc	ccc	gag	gtg	acc	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gag	gac	816
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
			260				265						270			
ccc	gag	gtg	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cac	aac	864
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
		275					280					285				
gcc	aag	acc	aag	ccc	cgg	gag	gag	cag	tac	aac	agc	acc	tac	cgg	gtg	912
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
	290					295					300					
gtg	agc	gtg	ctc	acc	gtg	ctg	cac	cag	gac	tgg	ctg	aac	ggc	aag	gag	960
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	
305					310					315					320	
tac	aag	tgc	aag	gtg	agc	aac	aag	gcc	ctg	cct	gcc	ccc	atc	gag	aag	1008
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	
				325					330					335		
acc	atc	agc	aag	gcc	aag	ggc	cag	ccc	cgg	gag	ccc	cag	gtg	tac	acc	1056
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
			340				345						350			
ctg	ccc	ccc	agc	cgg	gag	gag	atg	acc	aag	aac	cag	gtg	tcc	ctc	acc	1104
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	
			355				360					365				
tgt	ctg	gtg	aag	ggc	ttc	tac	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	1152
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	
	370					375					380					

50

```

agc aac ggc cag ccc gag aac aac tac aag acc acc ccc cct gtg ctg      1200
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                               390                               395                               400

```

```

gac agc gac ggc agc ttc ttc ctg tac agc aag ctc acc gtg gac aag      1248
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                               405                               410                               415

```

```

agc cgg tgg cag cag ggc aac gtg ttc agc tgc agc gtg atg cac gag      1296
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
                               420                               425                               430

```

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gcc ctg cac aac cac tac acc cag aag agc ctg agc ctg agc ccc ggc      1344
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                               435                               440                               445

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aag                                                                1347
Lys

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<210> 109
<211> 449
<212> PRT
<213> Artificial sequence

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<220>
<223> IgG heavy chain of CR03-022

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<400> 109

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

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Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Gly Phe Ile Thr Tyr
20                               25                               30

```

```

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35                               40                               45

```

```

Gly Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro Ser Phe
50                               55                               60

```

```

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Asn Thr Ala Tyr
65                               70                               75                               80

```

```

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile Tyr Tyr Cys
85                               90                               95

```

```

Ala Gly Gly Ser Gly Ile Ser Thr Pro Met Asp Val Trp Gly Gln Gly
100                               105                               110

```

```

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe

```

51

115					120					125					
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
130					135					140					
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
145				150					155						160
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
				165					170					175	
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180				185						190		
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	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	Glu	Glu	Met	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

52

				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

<210> 110
 <211> 642
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(642)
 <223> IgG light chain of CR03-014

<400> 110
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 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 gac aga gtc acc atc act tgc cgg gca agt cag agc att agc agc tac 96
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
 20 25 30
 tta aat tgg tat cag cag aaa cca ggg aaa gcc cct aag ctc ctg atc 144
 Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45
 tat gct gca tcc agt ttg caa agt ggg gtc cca tca agg ttc agt ggc 192
 Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 agt gga tct ggg aca gat ttc act ctc acc atc agc agt ctg caa cct 240
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 gaa gat ttt gca act tac tac tgt caa cag agt tac agt acc cct cca 288
 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Pro
 85 90 95
 acg ttc ggc caa ggg acc aag gtg gag atc aaa cgg acc gtg gcc gct 336
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 ccc agc gtg ttc atc ttc ccc ccc tcc gac gag cag ctg aag agc ggc 384
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125

```
acc gcc agc gtg gtg tgc ctg ctg aac aac ttc tac ccc cgg gag gcc      432
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
      130                      135                      140
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aag gtg cag tgg aag gtg gac aac gcc ctg cag agc ggc aac agc cag 480
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

gag agc gtg acc gag cag gac agc aag gac tcc acc tac agc ctg agc 528
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

agc acc ctc acc ctg agc aag gcc gac tac gag aag cac aag gtg tac 576
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

gcc tgc gag gtg acc cac cag ggc ctg agc agc ccc gtg acc aag agc 624
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

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ttc aac cgg ggc gag tgt
Phe Asn Arg Gly Glu Cys
      210

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<210>	111
<211>	214
<212>	PRT
<213>	Artificial sequence

<220>
<223> IgG light chain of CR03-014

<400> 111

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

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

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

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

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

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

54

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> 112
 <211> 660
 <212> DNA
 <213> Artificial sequence

<220>
 <221> CDS
 <222> (1)..(660)
 <223> IgG light chain of CR03-022

<400> 112
 gac atc gtg atg acc cag tct cca gac tcc ctg gct gtg tct ctg ggc 48
 Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 gag agg gcc acc atc aac tgc aag tcc agc cag agt gtt tta tac agc 96
 Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30
 tcc atc aat aag aac tac tta gct tgg tac cag cag aaa cca gga cag 144
 Ser Ile Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45
 cct cct aag ctg ctc att tac tgg gca tct acc cgg gaa tcc ggg gtc 192
 Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60
 cct gac cga ttc agt ggc agc ggg tct ggg aca gat ttc act ctc acc 240
 Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

55

65		70		75		80	
atc agc agc ctg cag gct gaa gat gtg gca gtt tat tac tgt cag caa							288
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln							
		85		90		95	
tat tat agt act ccg tac act ttt ggc cag ggg acc aag gtg gag atc							336
Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile							
		100		105		110	
aag cgg acc gtg gcc gct ccc agc gtg ttc atc ttc ccc ccc tcc gac							384
Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp							
		115		120		125	
gag cag ctg aag agc ggc acc gcc agc gtg gtg tgc ctg ctg aac aac							432
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn							
		130		135		140	
ttc tac ccc cgg gag gcc aag gtg cag tgg aag gtg gac aac gcc ctg							480
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu							
		145		150		155	160
cag agc ggc aac agc cag gag agc gtg acc gag cag gac agc aag gac							528
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp							
		165		170		175	
tcc acc tac agc ctg agc agc acc ctc acc ctg agc aag gcc gac tac							576
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr							
		180		185		190	
gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag ggc ctg agc							624
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser							
		195		200		205	
agc ccc gtg acc aag agc ttc aac cgg ggc gag tgt							660
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys							
		210		215		220	

<210> 113

<211> 220

<212> PRT

<213> Artificial sequence

<220>

<223> IgG light chain of CR03-022

<400> 113

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
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Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

56

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

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

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

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

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
145 150 155 160

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

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
180 185 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
195 200 205

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

<210> 114

<211> 3789

<212> DNA

<213> Artificial sequence

<220>

<221> CDS

<222> (10)..(3777)

<223> S protein of SARS-CoV strain Frankfurt 1

<400> 114

ggtaccgcc atg ttc atc ttc ctg ctg ttc ctg acc ctg acc agc ggc agc 51
Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser
1 5 10

gat ctg gat agg tgc acc acc ttc gac gac gtg cag gcc cct aat tac 99

57

Asp 15	Leu	Asp	Arg	Cys	Thr 20	Thr	Phe	Asp	Asp	Val 25	Gln	Ala	Pro	Asn	Tyr 30	
acc	cag	cac	acc	agc	tct	atg	cgg	ggc	gtg	tac	tac	ccc	gac	gag	atc	147
Thr	Gln	His	Thr	Ser 35	Ser	Met	Arg	Gly	Val 40	Tyr	Tyr	Pro	Asp	Glu 45	Ile	
ttc	aga	agc	gac	acc	ctg	tac	ctg	aca	cag	gac	ctg	ttc	ctg	ccc	ttc	195
Phe	Arg	Ser	Asp	Thr 50	Leu	Tyr	Leu	Thr 55	Gln	Asp	Leu	Phe	Leu 60	Pro	Phe	
tac	agc	aac	gtg	acc	ggc	ttc	cac	acc	atc	aac	cac	acc	ttc	ggc	aac	243
Tyr	Ser	Asn 65	Val	Thr	Gly	Phe	His 70	Thr	Ile	Asn	His	Thr 75	Phe	Gly	Asn	
ccc	gtg	atc	cct	ttc	aag	gac	ggc	atc	tac	ttc	gcc	gcc	acc	gag	aag	291
Pro	Val 80	Ile	Pro	Phe	Lys	Asp 85	Gly	Ile	Tyr	Phe	Ala 90	Ala	Thr	Glu	Lys	
agc	aat	gtg	gtg	cgg	ggc	tgg	gtg	ttc	ggc	agc	acc	atg	aac	aac	aag	339
Ser 95	Asn	Val	Val	Arg	Gly 100	Trp	Val	Phe	Gly	Ser 105	Thr	Met	Asn	Asn	Lys 110	
agc	cag	agc	gtg	atc	atc	atc	aac	aat	agc	acc	aac	gtg	gtg	atc	agg	387
Ser	Gln	Ser	Val	Ile 115	Ile	Ile	Asn	Asn	Ser 120	Thr	Asn	Val	Val	Ile 125	Arg	
gcc	tgc	aac	ttc	gag	ctg	tgc	gac	aac	cct	ttc	ttc	gcc	gtg	tcc	aaa	435
Ala	Cys	Asn	Phe 130	Glu	Leu	Cys	Asp	Asn 135	Pro	Phe	Phe	Ala	Val 140	Ser	Lys	
cct	atg	ggc	acc	cag	acc	cac	acc	atg	atc	ttc	gac	aac	gcc	ttc	aac	483
Pro	Met	Gly 145	Thr	Gln	Thr	His	Thr 150	Met	Ile	Phe	Asp	Asn 155	Ala	Phe	Asn	
tgc	acc	ttc	gag	tac	atc	agc	gac	gcc	ttc	agc	ctg	gat	gtg	agc	gag	531
Cys	Thr 160	Phe	Glu	Tyr	Ile	Ser 165	Asp	Ala	Phe	Ser	Leu 170	Asp	Val	Ser	Glu	
aag	agc	ggg	aac	ttc	aag	cac	ctg	cgg	gag	ttc	gtg	ttc	aag	aac	aag	579
Lys 175	Ser	Gly	Asn	Phe 180	Lys	His	Leu	Arg	Glu	Phe 185	Val	Phe	Lys	Asn	Lys 190	
gac	ggc	ttc	ctg	tac	gtg	tac	aag	ggc	tac	cag	ccc	atc	gac	gtg	gtg	627
Asp	Gly	Phe	Leu 195	Tyr	Val	Tyr	Lys	Gly	Tyr 200	Gln	Pro	Ile	Asp	Val 205	Val	
aga	gat	ctg	ccc	agc	ggc	ttc	aac	acc	ctg	aag	ccc	atc	ttc	aag	ctg	675
Arg	Asp	Leu 210	Pro	Ser	Gly	Phe	Asn 215	Thr	Leu	Lys	Pro	Ile	Phe 220	Lys	Leu	
ccc	ctg	ggc	atc	aac	atc	acc	aac	ttc	cgg	gcc	atc	ctg	acc	gcc	ttc	723
Pro	Leu 225	Gly	Ile	Asn	Ile	Thr	Asn 230	Phe	Arg	Ala	Ile	Leu 235	Thr	Ala	Phe	

58

agc cct gcc cag gac atc tgg ggc acc agc gcc gct gcc tac ttc gtg Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val 240 245 250	771
ggc tac ctg aag ccc acc acc ttc atg ctg aag tac gac gag aac ggc Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly 255 260 265 270	819
acc atc acc gat gcc gtg gac tgc agc cag aac ccc ctg gcc gag ctg Thr Ile Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu 275 280 285	867
aag tgc agc gtg aag agc ttc gag atc gac aag ggc atc tac cag acc Lys Cys Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr 290 295 300	915
agc aac ttc aga gtg gtg ccc agc ggc gat gtg gtg agg ttc ccc aac Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn 305 310 315	963
atc acc aac ctg tgc cct ttc ggc gag gtg ttc aac gcc acc aag ttc Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe 320 325 330	1011
cct agc gtg tac gcc tgg gag cgg aag aag atc agc aac tgc gtg gcc Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala 335 340 345 350	1059
gat tac agc gtg ctg tac aac agc acc ttc ttc agc acc ttc aag tgc Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys 355 360 365	1107
tac ggc gtg agc gcc acc aag ctg aac gac ctg tgc ttc agc aac gtg Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val 370 375 380	1155
tac gcc gac agc ttc gtg gtg aag ggc gac gac gtg aga cag atc gcc Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala 385 390 395	1203
cct ggc cag acc ggc gtg atc gcc gac tac aat tac aag ctg ccc gac Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp 400 405 410	1251
gac ttc atg ggc tgc gtg ctg gcc tgg aac acc aga aac atc gac gcc Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala 415 420 425 430	1299
acc tcc acc ggc aac tac aac tac aag tac cgc tac ctg agg cac ggc Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly 435 440 445	1347
aag ctg aga ccc ttc gag cgg gac atc agc aac gtg ccc ttc agc cct	1395

59

Lys	Leu	Arg	Pro	Phe	Glu	Arg	Asp	Ile	Ser	Asn	Val	Pro	Phe	Ser	Pro	
			450					455					460			
gac	ggc	aag	ccc	tgc	acc	ccc	cct	gcc	ctg	aac	tgc	tac	tgg	ccc	ctg	1443
Asp	Gly	Lys	Pro	Cys	Thr	Pro	Pro	Ala	Leu	Asn	Cys	Tyr	Trp	Pro	Leu	
		465					470					475				
aac	gac	tac	ggc	ttc	tac	acc	acc	acc	ggc	atc	ggc	tac	cag	cct	tac	1491
Asn	Asp	Tyr	Gly	Phe	Tyr	Thr	Thr	Thr	Gly	Ile	Gly	Tyr	Gln	Pro	Tyr	
	480					485					490					
aga	gtg	gtg	gtg	ctg	agc	ttc	gag	ctg	ctg	aac	gcc	cct	gcc	acc	gtg	1539
Arg	Val	Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr	Val	
495					500					505					510	
tgc	ggc	ccc	aag	ctg	agc	acc	gac	ctg	atc	aag	aac	cag	tgc	gtg	aac	1587
Cys	Gly	Pro	Lys	Leu	Ser	Thr	Asp	Leu	Ile	Lys	Asn	Gln	Cys	Val	Asn	
				515					520					525		
ttc	aac	ttc	aac	ggc	ctg	acc	ggc	acc	ggc	gtg	ctg	acc	cct	agc	agc	1635
Phe	Asn	Phe	Asn	Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Pro	Ser	Ser	
			530					535					540			
aag	agg	ttc	cag	ccc	ttc	cag	cag	ttc	ggc	agg	gac	gtg	agc	gat	ttc	1683
Lys	Arg	Phe	Gln	Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Val	Ser	Asp	Phe	
		545					550					555				
acc	gac	agc	gtg	agg	gat	cct	aag	acc	agc	gag	atc	ctg	gac	atc	agc	1731
Thr	Asp	Ser	Val	Arg	Asp	Pro	Lys	Thr	Ser	Glu	Ile	Leu	Asp	Ile	Ser	
	560					565					570					
cct	tgc	agc	ttc	ggc	ggc	gtg	agc	gtg	atc	acc	ccc	ggc	acc	aac	gcc	1779
Pro	Cys	Ser	Phe	Gly	Gly	Val	Ser	Val	Ile	Thr	Pro	Gly	Thr	Asn	Ala	
575					580					585					590	
agc	tcc	gag	gtg	gcc	gtg	ctg	tac	cag	gac	gtg	aac	tgc	acc	gac	gtg	1827
Ser	Ser	Glu	Val	Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	Asp	Val	
				595				600						605		
agc	acc	gcc	atc	cac	gcc	gac	cag	ctg	acc	ccc	gcc	tgg	aga	atc	tac	1875
Ser	Thr	Ala	Ile	His	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	Ile	Tyr	
			610					615					620			
agc	acc	ggc	aac	aac	gtg	ttc	cag	acc	cag	gcc	ggc	tgc	ctg	atc	ggc	1923
Ser	Thr	Gly	Asn	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	Ile	Gly	
		625					630					635				
gcc	gag	cac	gtg	gac	acc	agc	tac	gag	tgc	gac	atc	ccc	atc	gga	gcc	1971
Ala	Glu	His	Val	Asp	Thr	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	
	640					645					650					
ggc	atc	tgc	gcc	agc	tac	cac	acc	gtg	agc	ctg	ctg	aga	agc	acc	agc	2019
Gly	Ile	Cys	Ala	Ser	Tyr	His	Thr	Val	Ser	Leu	Leu	Arg	Ser	Thr	Ser	
655					660					665					670	

60

cag aag agc atc gtg gcc tac acc atg agc ctg ggc gcc gac agc agc	2067
Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser	
675 680 685	
atc gcc tac agc aac aac acc atc gcc atc ccc acc aac ttc agc atc	2115
Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile	
690 695 700	
agc atc acc acc gag gtg atg ccc gtg agc atg gcc aag acc agc gtg	2163
Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val	
705 710 715	
gac tgc aac atg tac atc tgc ggc gac agc acc gag tgc gcc aac ctg	2211
Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu	
720 725 730	
ctg ctg cag tac ggc agc ttc tgc acc cag ctg aac aga gcc ctg agc	2259
Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser	
735 740 745 750	
ggc atc gcc gcc gag cag gac aga aac acc agg gag gtg ttc gcc cag	2307
Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln	
755 760 765	
gtg aag cag atg tat aag acc ccc acc ctg aag tac ttc ggc ggc ttc	2355
Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe	
770 775 780	
aac ttc agc cag atc ctg ccc gat cct ctg aag ccc acc aag cgg agc	2403
Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser	
785 790 795	
ttc atc gag gac ctg ctg ttc aac aag gtg acc ctg gcc gac gcc ggc	2451
Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly	
800 805 810	
ttt atg aag cag tac ggc gag tgc ctg ggc gat atc aac gcc agg gac	2499
Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp	
815 820 825 830	
ctg atc tgc gcc cag aag ttc aat ggc ctg acc gtg ctg ccc ccc ctg	2547
Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu	
835 840 845	
ctg acc gac gac atg atc gcc gcc tac aca gcc gcc ctg gtg agc ggc	2595
Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly	
850 855 860	
acc gcc acc gcc ggc tgg acc ttt ggc gcc gga gcc gcc ctg cag atc	2643
Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile	
865 870 875	
ccc ttc gcc atg cag atg gcc tac cgg ttc aat ggc atc ggc gtg acc	2691

61

Pro	Phe	Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr		
880						885					890						
cag	aac	gtg	ctg	tac	gag	aac	cag	aag	cag	atc	gcc	aac	cag	ttc	aac	2739	
Gln	Asn	Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	Phe	Asn		
895					900					905					910		
aag	gcc	atc	agc	cag	atc	cag	gag	agc	ctg	acc	acc	aca	agc	acc	gcc	2787	
Lys	Ala	Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	Thr	Ala		
				915					920					925			
ctg	ggc	aag	ctg	cag	gac	gtg	gtg	aac	cag	aac	gcc	cag	gcc	ctg	aat	2835	
Leu	Gly	Lys	Leu	Gln	Asp	Val	Val	Asn	Gln	Asn	Ala	Gln	Ala	Leu	Asn		
			930					935					940				
acc	ctg	gtg	aag	cag	ctg	agc	agc	aac	ttc	ggc	gcc	atc	agc	tcc	gtg	2883	
Thr	Leu	Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	Ser	Val		
		945					950					955					
ctg	aac	gac	atc	ctg	agc	cgg	ctg	gac	aag	gtg	gag	gcc	gag	gtg	cag	2931	
Leu	Asn	Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	Val	Gln		
	960					965					970						
atc	gac	aga	ctg	atc	acc	ggc	aga	ctg	cag	agc	ctg	cag	acc	tac	gtg	2979	
Ile	Asp	Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	Tyr	Val		
975					980					985					990		
acc	cag	cag	ctg	atc	aga	gcc	gcc	gag	atc	aga	gcc	agc	gcc	aac	ctg	3027	
Thr	Gln	Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile	Arg	Ala	Ser	Ala	Asn	Leu		
				995					1000					1005			
gcc	gcc	acc	aag	atg	agc	gag	tgc	gtg	ctg	ggc	cag	agc	aag	aga		3072	
Ala	Ala	Thr	Lys	Met	Ser	Glu	Cys	Val	Leu	Gly	Gln	Ser	Lys	Arg			
			1010					1015					1020				
gtg	gac	ttc	tgc	ggc	aag	ggc	tac	cac	ctg	atg	agc	ttc	ccc	cag		3117	
Val	Asp	Phe	Cys	Gly	Lys	Gly	Tyr	His	Leu	Met	Ser	Phe	Pro	Gln			
			1025					1030					1035				
gcc	gct	ccc	cac	ggc	gtg	gtg	ttc	ctg	cac	gtg	acc	tac	gtg	cct		3162	
Ala	Ala	Pro	His	Gly	Val	Val	Phe	Leu	His	Val	Thr	Tyr	Val	Pro			
			1040					1045					1050				
agc	cag	gag	agg	aat	ttc	acc	acc	gcc	cct	gcc	atc	tgc	cac	gag		3207	
Ser	Gln	Glu	Arg	Asn	Phe	Thr	Thr	Ala	Pro	Ala	Ile	Cys	His	Glu			
			1055					1060					1065				
ggc	aag	gcc	tac	ttc	ccc	aga	gag	ggc	gtg	ttc	gtg	ttc	aat	ggc		3252	
Gly	Lys	Ala	Tyr	Phe	Pro	Arg	Glu	Gly	Val	Phe	Val	Phe	Asn	Gly			
			1070					1075					1080				
acc	agc	tgg	ttc	atc	acc	cag	cgg	aac	ttc	ttc	agc	ccc	cag	atc		3297	
Thr	Ser	Trp	Phe	Ile	Thr	Gln	Arg	Asn	Phe	Phe	Ser	Pro	Gln	Ile			
			1085					1090					1095				

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atc	aca	acc	gac	aac	acc	ttc	gtg	agc	ggc	aac	tgc	gac	gtg	gtg	3342
Ile	Thr	Thr	Asp	Asn	Thr	Phe	Val	Ser	Gly	Asn	Cys	Asp	Val	Val	
			1100					1105					1110		
atc	ggc	atc	att	aac	aat	acc	gtg	tac	gac	ccc	ctg	cag	ccc	gag	3387
Ile	Gly	Ile	Ile	Asn	Asn	Thr	Val	Tyr	Asp	Pro	Leu	Gln	Pro	Glu	
			1115					1120					1125		
ctg	gat	agc	ttc	aag	gag	gag	ctg	gac	aag	tac	ttc	aag	aac	cac	3432
Leu	Asp	Ser	Phe	Lys	Glu	Glu	Leu	Asp	Lys	Tyr	Phe	Lys	Asn	His	
			1130					1135					1140		
acc	agc	ccc	gat	gtg	gac	ttc	ggc	gac	atc	agc	ggc	atc	aat	gcc	3477
Thr	Ser	Pro	Asp	Val	Asp	Phe	Gly	Asp	Ile	Ser	Gly	Ile	Asn	Ala	
			1145					1150					1155		
agc	gtg	gtg	aac	atc	cag	aag	gag	atc	gac	cgg	ctg	aac	gag	gtg	3522
Ser	Val	Val	Asn	Ile	Gln	Lys	Glu	Ile	Asp	Arg	Leu	Asn	Glu	Val	
			1160					1165					1170		
gcc	aag	aac	ctg	aac	gag	agc	ctg	atc	gac	ctg	cag	gag	ctg	ggc	3567
Ala	Lys	Asn	Leu	Asn	Glu	Ser	Leu	Ile	Asp	Leu	Gln	Glu	Leu	Gly	
			1175					1180					1185		
aag	tac	gag	cag	tac	atc	aag	tgg	ccc	tgg	tac	gtg	tgg	ctg	ggc	3612
Lys	Tyr	Glu	Gln	Tyr	Ile	Lys	Trp	Pro	Trp	Tyr	Val	Trp	Leu	Gly	
			1190					1195					1200		
ttc	atc	gcc	ggc	ctg	atc	gcc	atc	gtg	atg	gtg	acc	atc	ctg	ctg	3657
Phe	Ile	Ala	Gly	Leu	Ile	Ala	Ile	Val	Met	Val	Thr	Ile	Leu	Leu	
			1205					1210					1215		
tgc	tgc	atg	acc	agc	tgc	tgc	tcc	tgc	ctg	aag	ggc	gcc	tgc	agc	3702
Cys	Cys	Met	Thr	Ser	Cys	Cys	Ser	Cys	Leu	Lys	Gly	Ala	Cys	Ser	
			1220					1225					1230		
tgt	ggc	agc	tgc	tgc	aag	ttc	gac	gag	gac	gat	agc	gag	ccc	gtg	3747
Cys	Gly	Ser	Cys	Cys	Lys	Phe	Asp	Glu	Asp	Asp	Ser	Glu	Pro	Val	
			1235					1240					1245		
ctg	aag	ggc	gtg	aag	ctg	cac	tac	acc	tga	tgaattctctcg	ag				3789
Leu	Lys	Gly	Val	Lys	Leu	His	Tyr	Thr							
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<211> 1255

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<223> S protein of SARS-CoV strain Frankfurt 1

63

<400> 115

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

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275					280					285					
Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn
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Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg	Phe	Pro	Asn	Ile	Thr
305					310					315					320
Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Lys	Phe	Pro	Ser
				325					330					335	
Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr
			340					345					350		
Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly
		355					360					365			
Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Ser	Asn	Val	Tyr	Ala
						375					380				
Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg	Gln	Ile	Ala	Pro	Gly
385					390					395					400
Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe
				405					410					415	
Met	Gly	Cys	Val	Leu	Ala	Trp	Asn	Thr	Arg	Asn	Ile	Asp	Ala	Thr	Ser
			420					425					430		
Thr	Gly	Asn	Tyr	Asn	Tyr	Lys	Tyr	Arg	Tyr	Leu	Arg	His	Gly	Lys	Leu
		435					440					445			
Arg	Pro	Phe	Glu	Arg	Asp	Ile	Ser	Asn	Val	Pro	Phe	Ser	Pro	Asp	Gly
						455					460				
Lys	Pro	Cys	Thr	Pro	Pro	Ala	Leu	Asn	Cys	Tyr	Trp	Pro	Leu	Asn	Asp
465					470					475					480
Tyr	Gly	Phe	Tyr	Thr	Thr	Thr	Gly	Ile	Gly	Tyr	Gln	Pro	Tyr	Arg	Val
				485					490					495	
Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr	Val	Cys	Gly
			500					505					510		
Pro	Lys	Leu	Ser	Thr	Asp	Leu	Ile	Lys	Asn	Gln	Cys	Val	Asn	Phe	Asn
		515					520					525			
Phe	Asn	Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Pro	Ser	Ser	Lys	Arg
		530				535					540				
Phe	Gln	Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Val	Ser	Asp	Phe	Thr	Asp
545				550					555						560
Ser	Val	Arg	Asp	Pro	Lys	Thr	Ser	Glu	Ile	Leu	Asp	Ile	Ser	Pro	Cys

65

565					570					575					
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			580					585					590		
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		595					600					605			
Ala	Ile	His	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	Ile	Tyr	Ser	Thr
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Gly	Asn	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	Ile	Gly	Ala	Glu
625						630					635				640
His	Val	Asp	Thr	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	Gly	Ile
				645					650					655	
Cys	Ala	Ser	Tyr	His	Thr	Val	Ser	Leu	Leu	Arg	Ser	Thr	Ser	Gln	Lys
			660					665					670		
Ser	Ile	Val	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Asp	Ser	Ser	Ile	Ala
		675					680					685			
Tyr	Ser	Asn	Asn	Thr	Ile	Ala	Ile	Pro	Thr	Asn	Phe	Ser	Ile	Ser	Ile
	690					695					700				
Thr	Thr	Glu	Val	Met	Pro	Val	Ser	Met	Ala	Lys	Thr	Ser	Val	Asp	Cys
705						710					715				720
Asn	Met	Tyr	Ile	Cys	Gly	Asp	Ser	Thr	Glu	Cys	Ala	Asn	Leu	Leu	Leu
				725					730					735	
Gln	Tyr	Gly	Ser	Phe	Cys	Thr	Gln	Leu	Asn	Arg	Ala	Leu	Ser	Gly	Ile
			740					745					750		
Ala	Ala	Glu	Gln	Asp	Arg	Asn	Thr	Arg	Glu	Val	Phe	Ala	Gln	Val	Lys
		755					760					765			
Gln	Met	Tyr	Lys	Thr	Pro	Thr	Leu	Lys	Tyr	Phe	Gly	Gly	Phe	Asn	Phe
	770					775					780				
Ser	Gln	Ile	Leu	Pro	Asp	Pro	Leu	Lys	Pro	Thr	Lys	Arg	Ser	Phe	Ile
785						790					795				800
Glu	Asp	Leu	Leu	Phe	Asn	Lys	Val	Thr	Leu	Ala	Asp	Ala	Gly	Phe	Met
				805					810					815	
Lys	Gln	Tyr	Gly	Glu	Cys	Leu	Gly	Asp	Ile	Asn	Ala	Arg	Asp	Leu	Ile
			820					825					830		
Cys	Ala	Gln	Lys	Phe	Asn	Gly	Leu	Thr	Val	Leu	Pro	Pro	Leu	Leu	Thr
		835					840					845			
Asp	Asp	Met	Ile	Ala	Ala	Tyr	Thr	Ala	Ala	Leu	Val	Ser	Gly	Thr	Ala

66

850		855		860											
Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ala	Ala	Leu	Gln	Ile	Pro	Phe
865					870				875						880
Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr	Gln	Asn
				885					890					895	
Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	Phe	Asn	Lys	Ala
			900					905					910		
Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	Thr	Ala	Leu	Gly
		915					920					925			
Lys	Leu	Gln	Asp	Val	Val	Asn	Gln	Asn	Ala	Gln	Ala	Leu	Asn	Thr	Leu
	930					935					940				
Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	Ser	Val	Leu	Asn
945					950					955					960
Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	Val	Gln	Ile	Asp
				965					970					975	
Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	Tyr	Val	Thr	Gln
			980					985						990	
Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile	Arg	Ala	Ser	Ala	Asn	Leu	Ala	Ala
		995					1000					1005			
Thr	Lys	Met	Ser	Glu	Cys	Val	Leu	Gly	Gln	Ser	Lys	Arg	Val	Asp	
	1010					1015					1020				
Phe	Cys	Gly	Lys	Gly	Tyr	His	Leu	Met	Ser	Phe	Pro	Gln	Ala	Ala	
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Pro	His	Gly	Val	Val	Phe	Leu	His	Val	Thr	Tyr	Val	Pro	Ser	Gln	
	1040					1045					1050				
Glu	Arg	Asn	Phe	Thr	Thr	Ala	Pro	Ala	Ile	Cys	His	Glu	Gly	Lys	
	1055					1060					1065				
Ala	Tyr	Phe	Pro	Arg	Glu	Gly	Val	Phe	Val	Phe	Asn	Gly	Thr	Ser	
	1070					1075					1080				
Trp	Phe	Ile	Thr	Gln	Arg	Asn	Phe	Phe	Ser	Pro	Gln	Ile	Ile	Thr	
	1085					1090					1095				
Thr	Asp	Asn	Thr	Phe	Val	Ser	Gly	Asn	Cys	Asp	Val	Val	Ile	Gly	
	1100					1105					1110				
Ile	Ile	Asn	Asn	Thr	Val	Tyr	Asp	Pro	Leu	Gln	Pro	Glu	Leu	Asp	
	1115					1120					1125				
Ser	Phe	Lys	Glu	Glu	Leu	Asp	Lys	Tyr	Phe	Lys	Asn	His	Thr	Ser	

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1130	1135	1140
Pro Asp Val Asp Phe Gly Asp Ile Ser Gly Ile Asn Ala Ser Val		
1145	1150	1155
Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys		
1160	1165	1170
Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr		
1175	1180	1185
Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp Leu Gly Phe Ile		
1190	1195	1200
Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu Leu Cys Cys		
1205	1210	1215
Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser Cys Gly		
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Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys		
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Gly Val Lys Leu His Tyr Thr		
1250	1255	

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<220>
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32

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<210> 118
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<222> (1)..(21)

<223> Region of S protein

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Pro Phe Ser Pro Asp Gly Lys

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21

<210> 119

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<211> 21

<212> DNA

<213> Artificial sequence

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<222> (1)..(21)

<223> Region of S protein

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<211> 7

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<400> 123
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 tacacacctc agcgttg 17

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<400> 125
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<210> 126
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Gly	Lys	Leu	Arg
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Ser	Phe	Glu	Leu
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<210> 128

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<223> X can be any amino acid

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<223> X can be any amino acid

71

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<400> 128

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<210> 129
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<400> 129

Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Pro	Thr
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Asp	His	Tyr	Met	Asp
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<223> HCDR2 of SC03-014

<400> 131

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Val Lys Gly

<210> 132

<211> 11

<212> PRT

<213> Artificial sequence

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<223> LCDR1 of SC03-014

<400> 132

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<211> 7

<212> PRT

<213> Artificial sequence

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<400> 133

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1				5		

<210> 134

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<212> PRT

<213> Artificial sequence

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Gln	Gln	Tyr	Tyr	Ser	Thr	Pro	Tyr	Thr
1				5				

73

<210> 135
 <211> 5
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<400> 135

Thr Tyr Trp Ile Gly
 1 5

<210> 136
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Ile Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro Ser Phe Gln
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Gly

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<400> 137

Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Ile Asn Lys Asn Tyr Leu
 1 5 10 15

Ala

<210> 138
 <211> 7
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<223> LCDR2 of SC03-022

<400> 138

Trp Ala Ser Thr Arg Glu Ser
1 5

CLAIMS:

1. A composition comprising at least two immunoglobulins that are capable of specifically binding to a SARS-CoV and have SARS-CoV neutralizing activity,

wherein the first immunoglobulin comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8 and;

wherein the second immunoglobulin comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10;

characterized in that the relative amounts of the immunoglobulins are such that they exhibit a synergistic neutralizing activity.

2. A composition according to claim 1, characterized in that the immunoglobulins are capable of reacting with different, non-competing epitopes of the SARS-CoV.

3. A composition according to claims 1 or 2, characterized in that the immunoglobulins are capable of reacting with different, non-competing epitopes of the S protein of SARS-CoV.

4. A composition according to any one of claims 1 to 3, characterized in that the immunoglobulins are capable of reacting with different, non-competing epitopes of amino acids 318-510 of the S protein of SARS-CoV.

5. A composition according to any one of claims 1 to 4, characterized in that at least one of the immunoglobulins reacts with an epitope comprising the amino acid sequence of SEQ ID NO:128.

6. A composition according to any one of claims 1 to 5, characterized in that at least one of the immunoglobulins is capable of reacting with amino acids 318-510 of the S protein of a human and an animal SARS-CoV.

7. A composition according to any one of claims 1 to 6, characterized in that at least one of the immunoglobulins is capable of reacting with amino acids 318-510 of the S protein of a SARS-CoV, wherein amino acid 479 is an amino acid other than asparagine, to a similar extent as with amino acids 318-510 of the S protein of a SARS-CoV, wherein amino acid 479 is an asparagine.

8. A composition according to any one of claims 1 to 7, characterized in that each of the immunoglobulins is capable of neutralizing a plurality of SARS-CoV strains.

9. A composition according to any one of claims 1 to 8, characterized in that the molar ratio of the two immunoglobulins is from 1:100 to 100:1.

10. A composition according to any one of claims 1 to 9, characterized in that a first immunoglobulin comprises at least a heavy chain CDR3 region comprising the amino acid sequence of SEQ ID NO:1 and a second immunoglobulin

comprises at least a heavy chain CDR3 region comprising the amino acid sequence of SEQ ID NO:2.

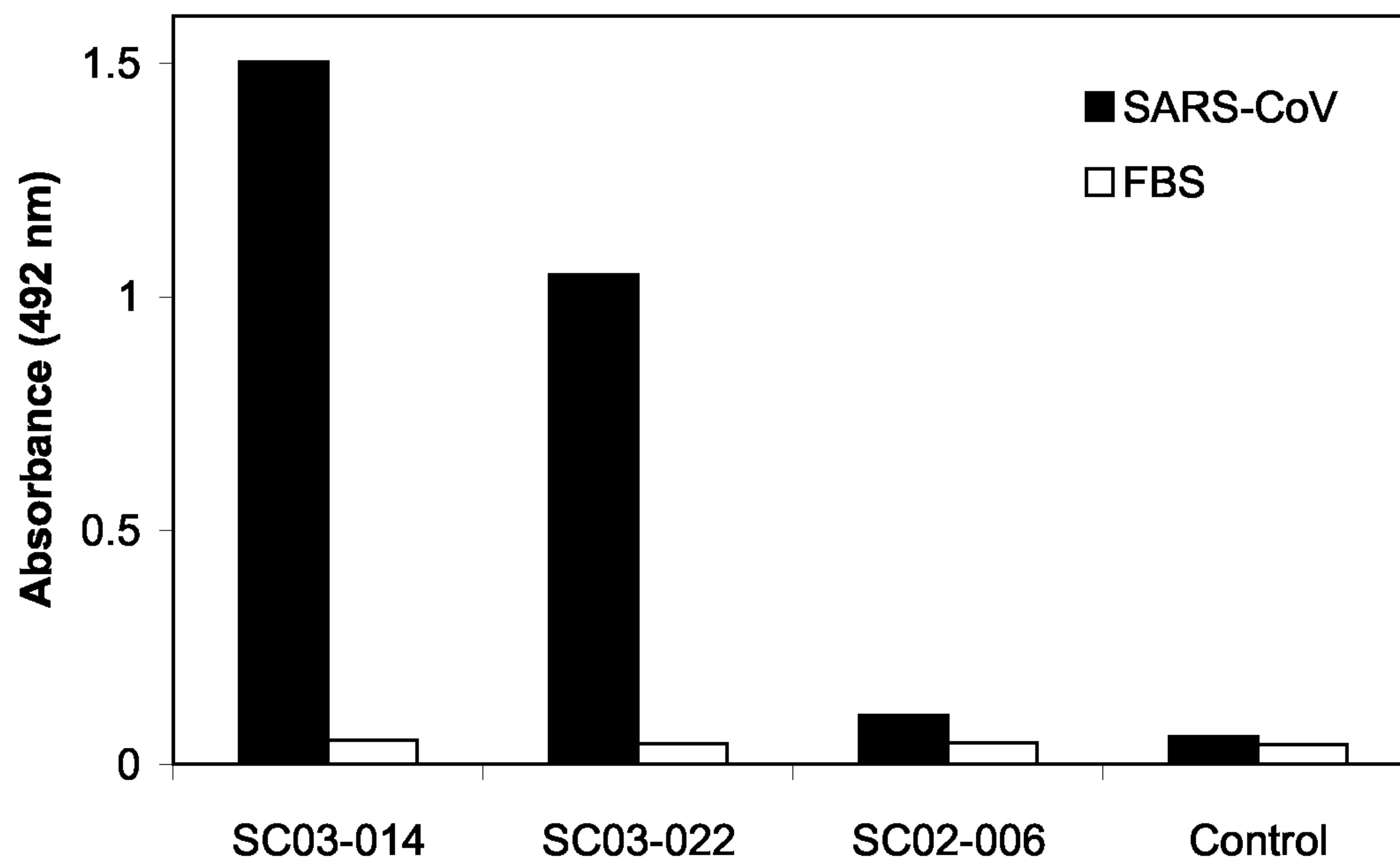
11. A pharmaceutical composition comprising a composition according to any one of claims 1 to 10, the pharmaceutical composition further comprising a pharmaceutically acceptable excipient.

12. A pharmaceutical composition according to claim 11 for use as a medicament against SARS-CoV.

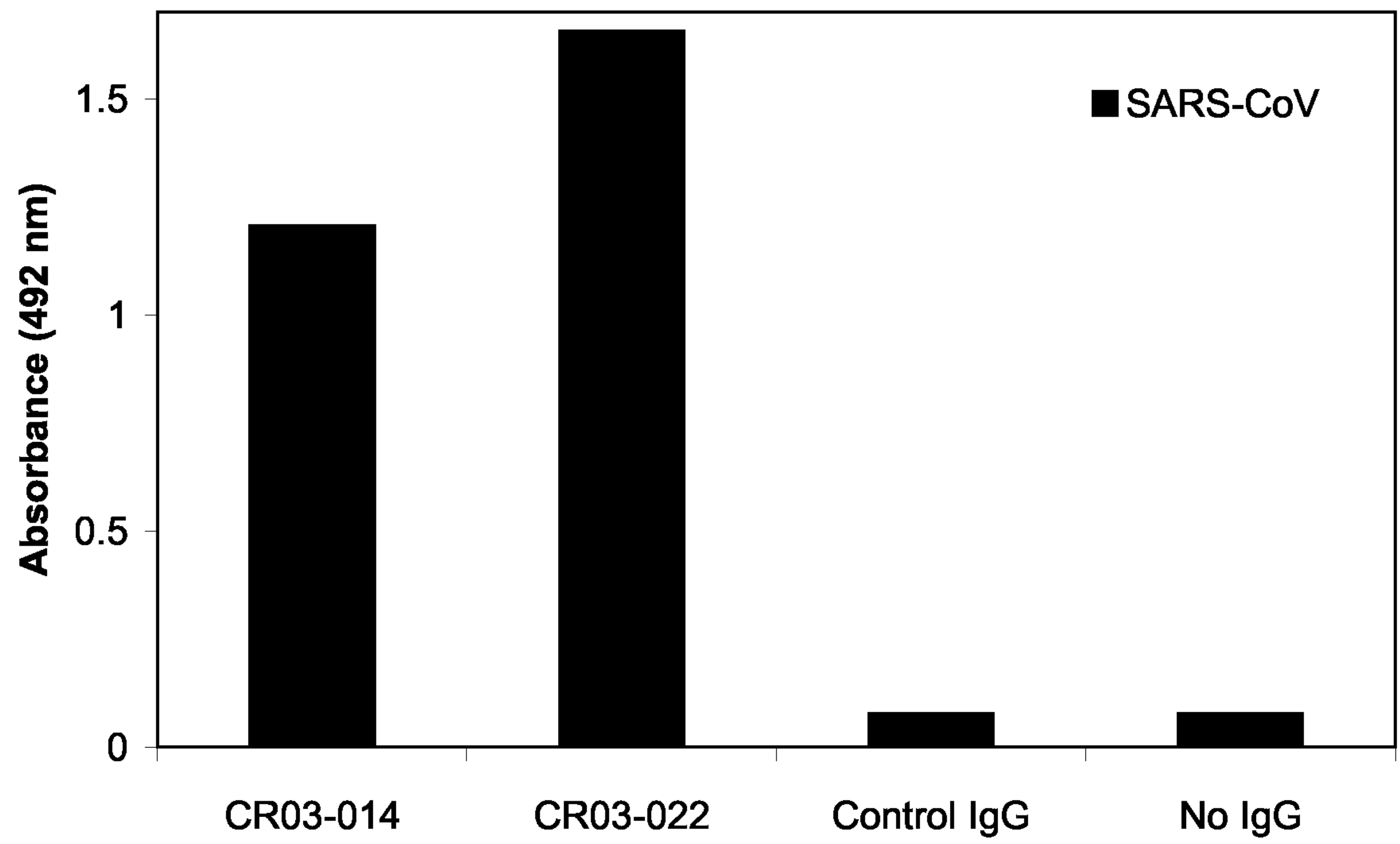
13. A pharmaceutical composition according to claim 11 for use in the diagnosis, prophylaxis, treatment, or combination thereof of a condition resulting from a SARS-CoV.

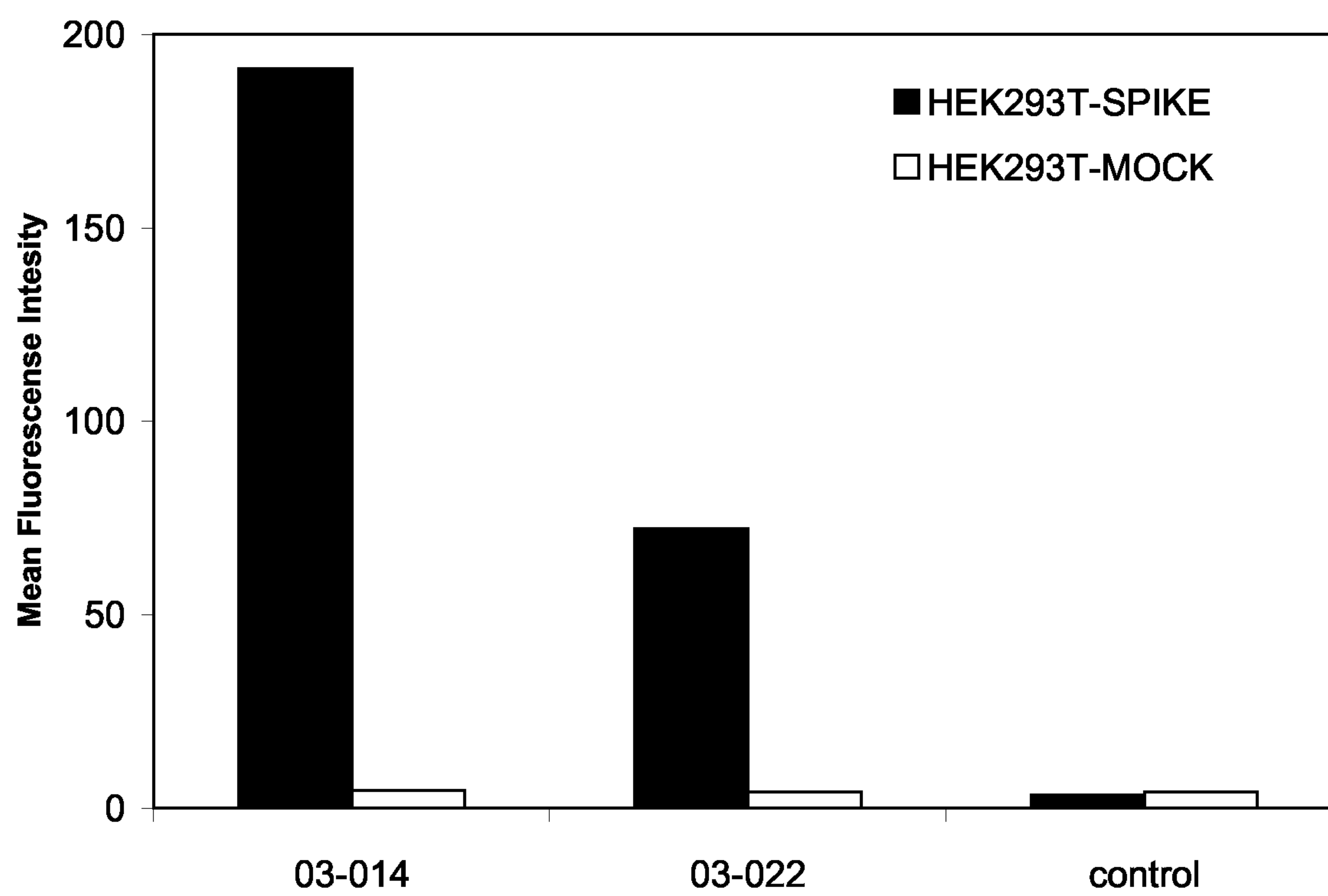
14. Use of a pharmaceutical composition according to claim 11 in the preparation of a medicament for the diagnosis, prophylaxis, treatment, or combination thereof of a condition resulting from a SARS-CoV.

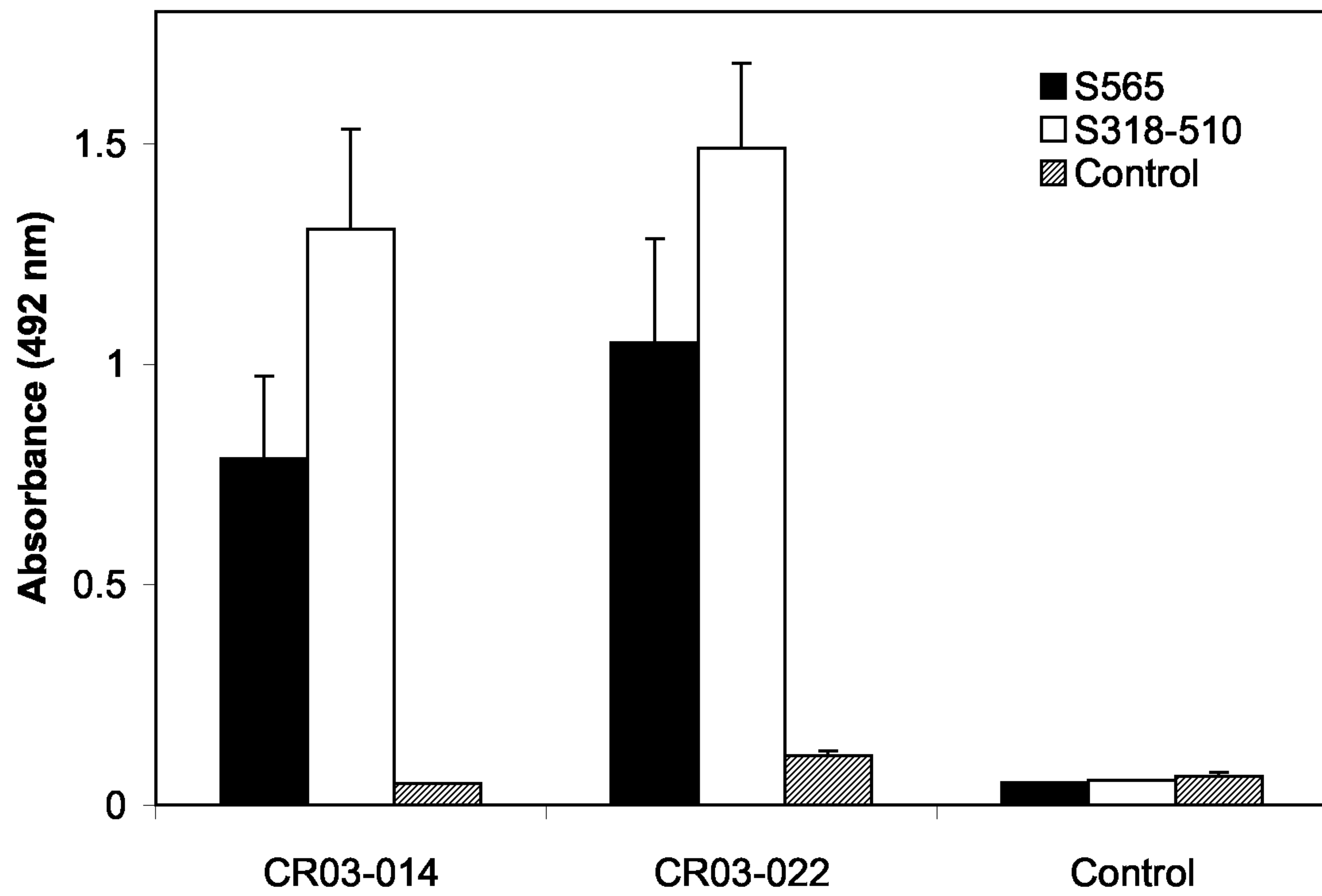
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**FIGURE 1**

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**FIGURE 2**

3/11**FIGURE 3**

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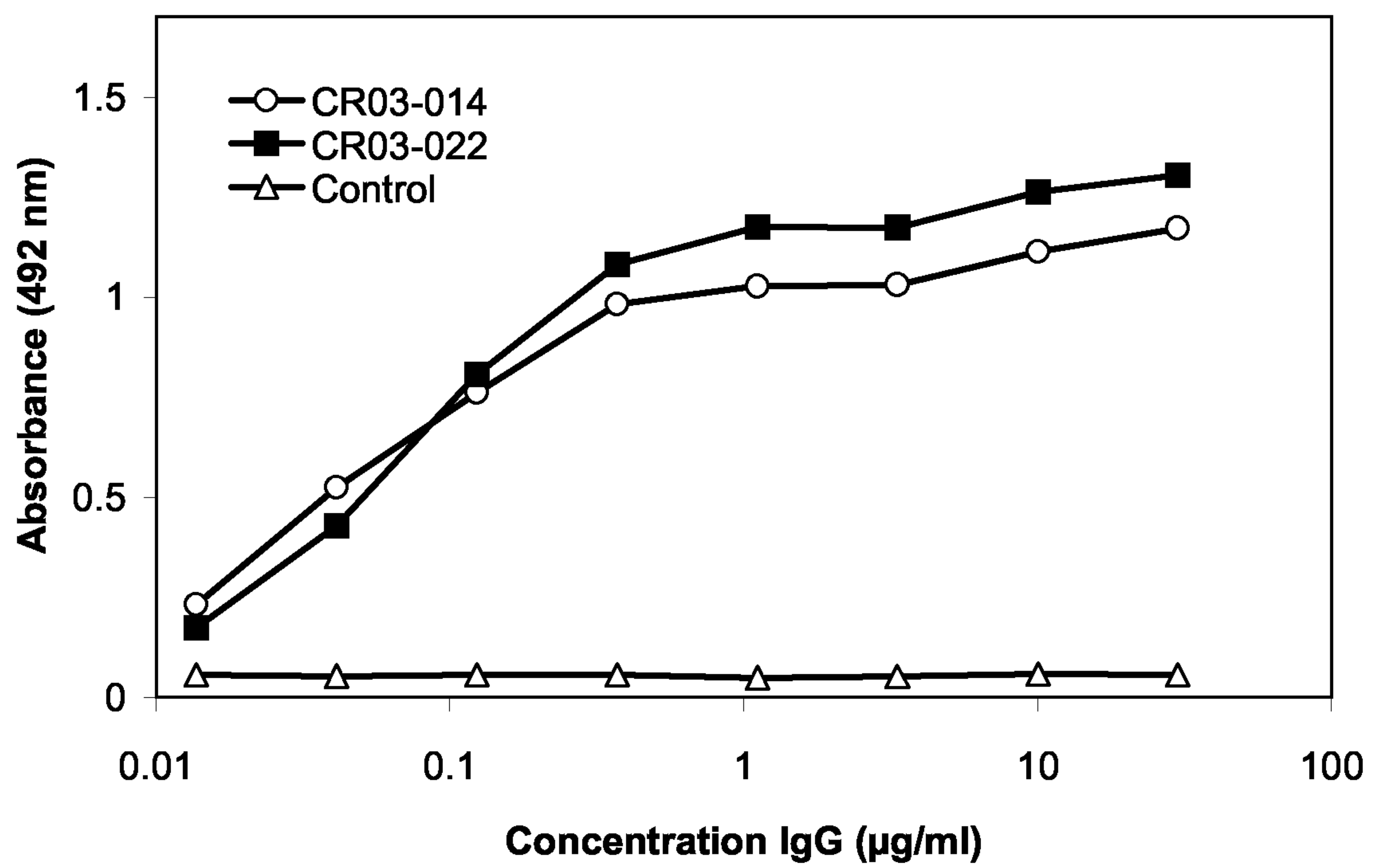


FIGURE 5

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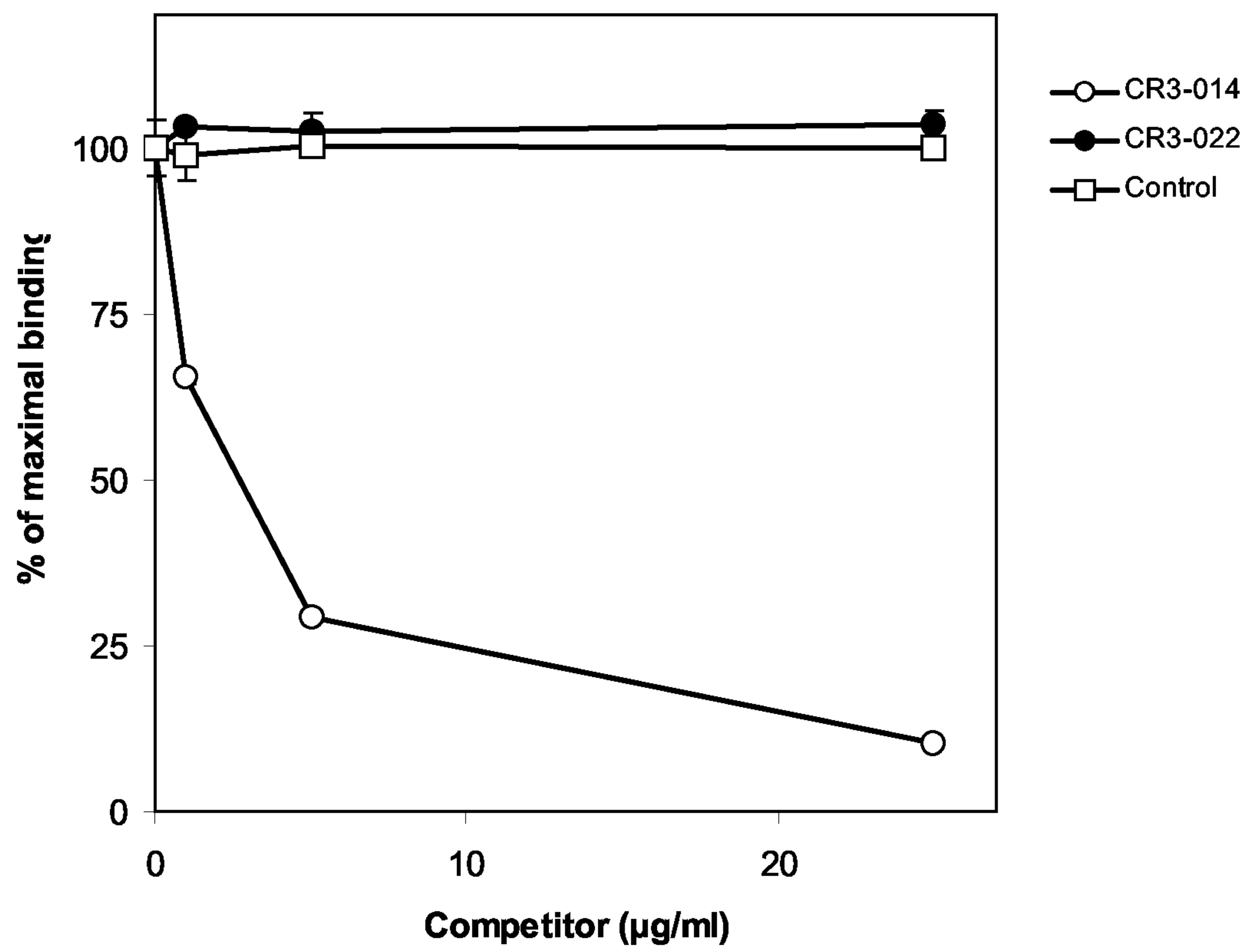


FIGURE 6A

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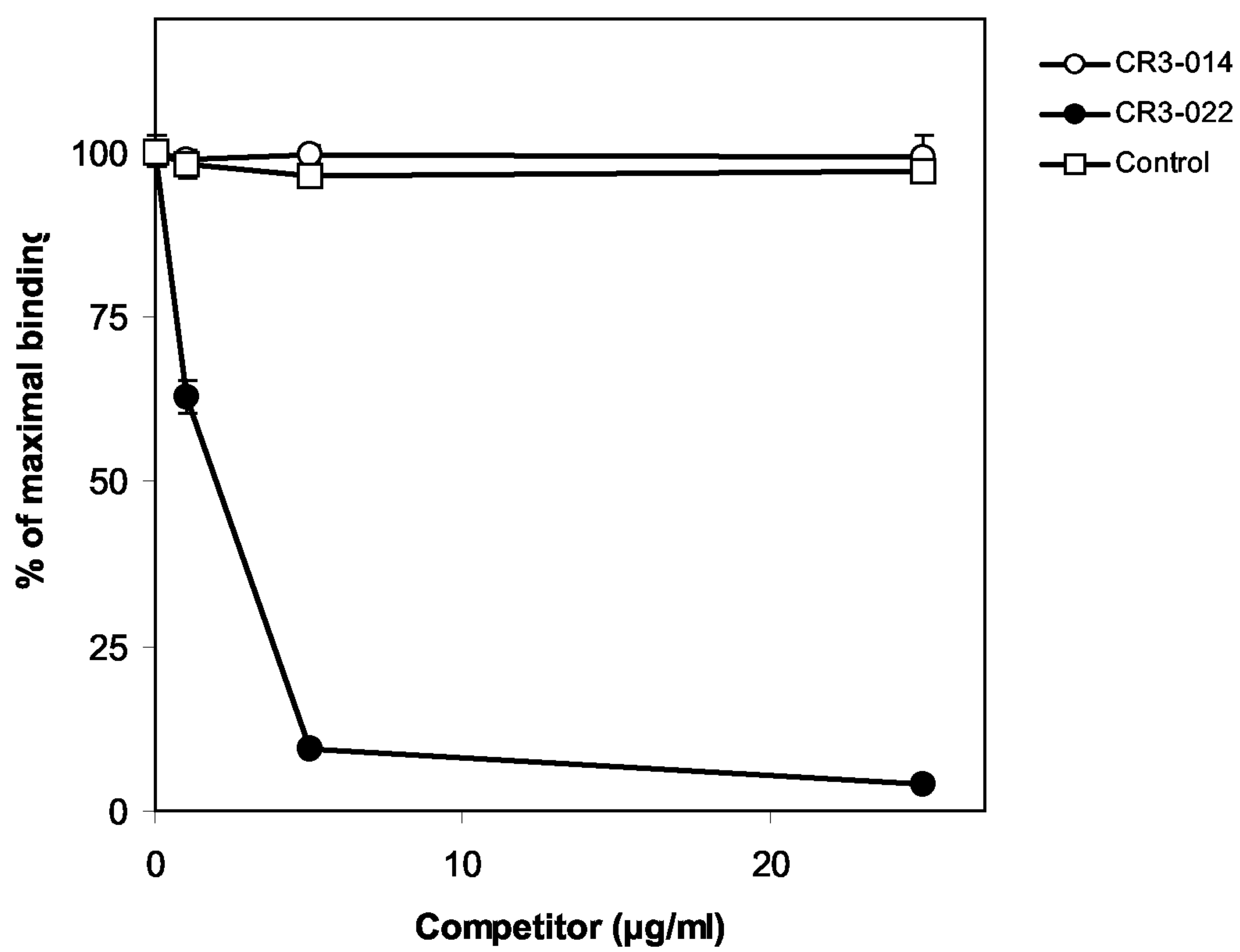
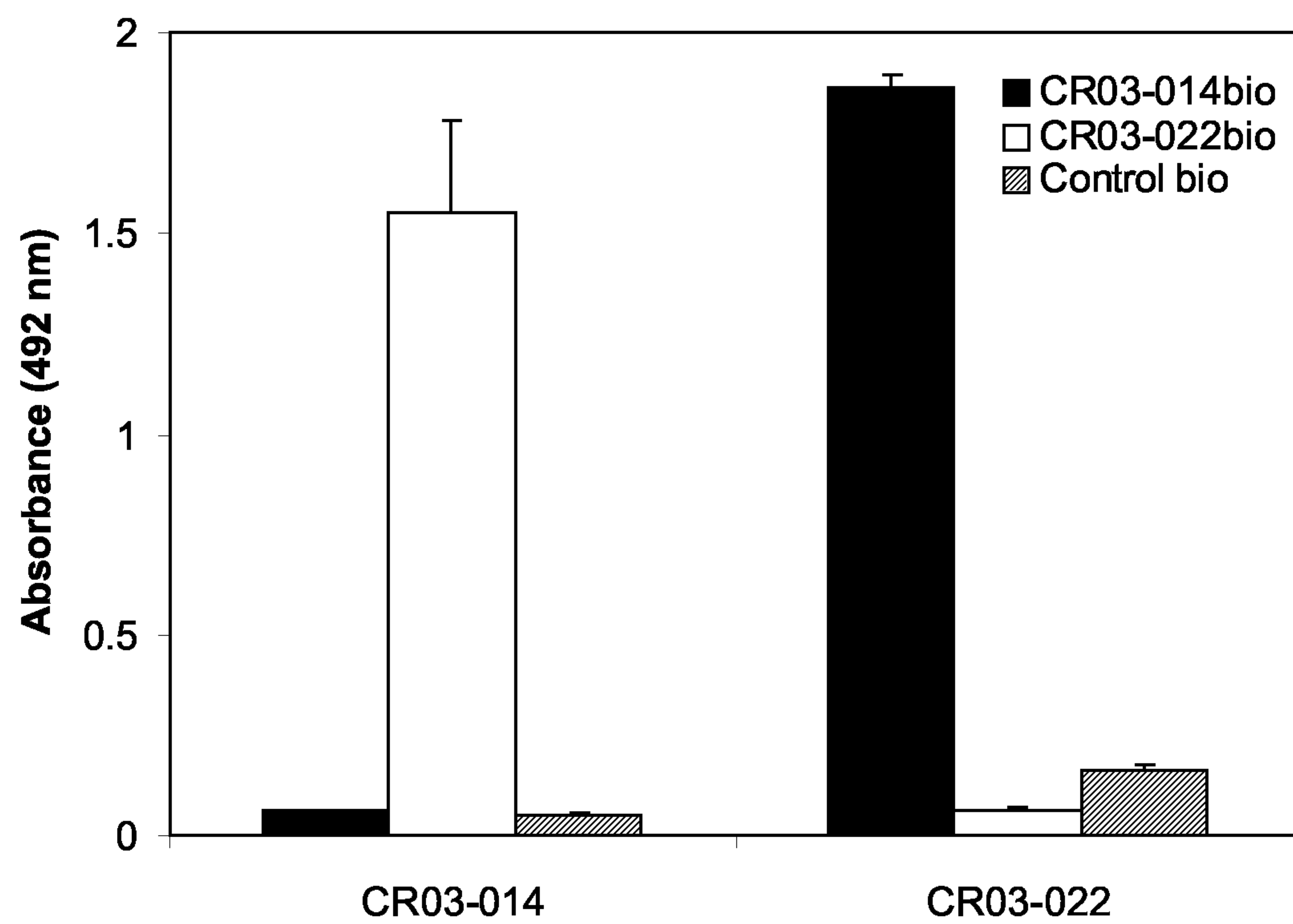


FIGURE 6B

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**FIGURE 7**

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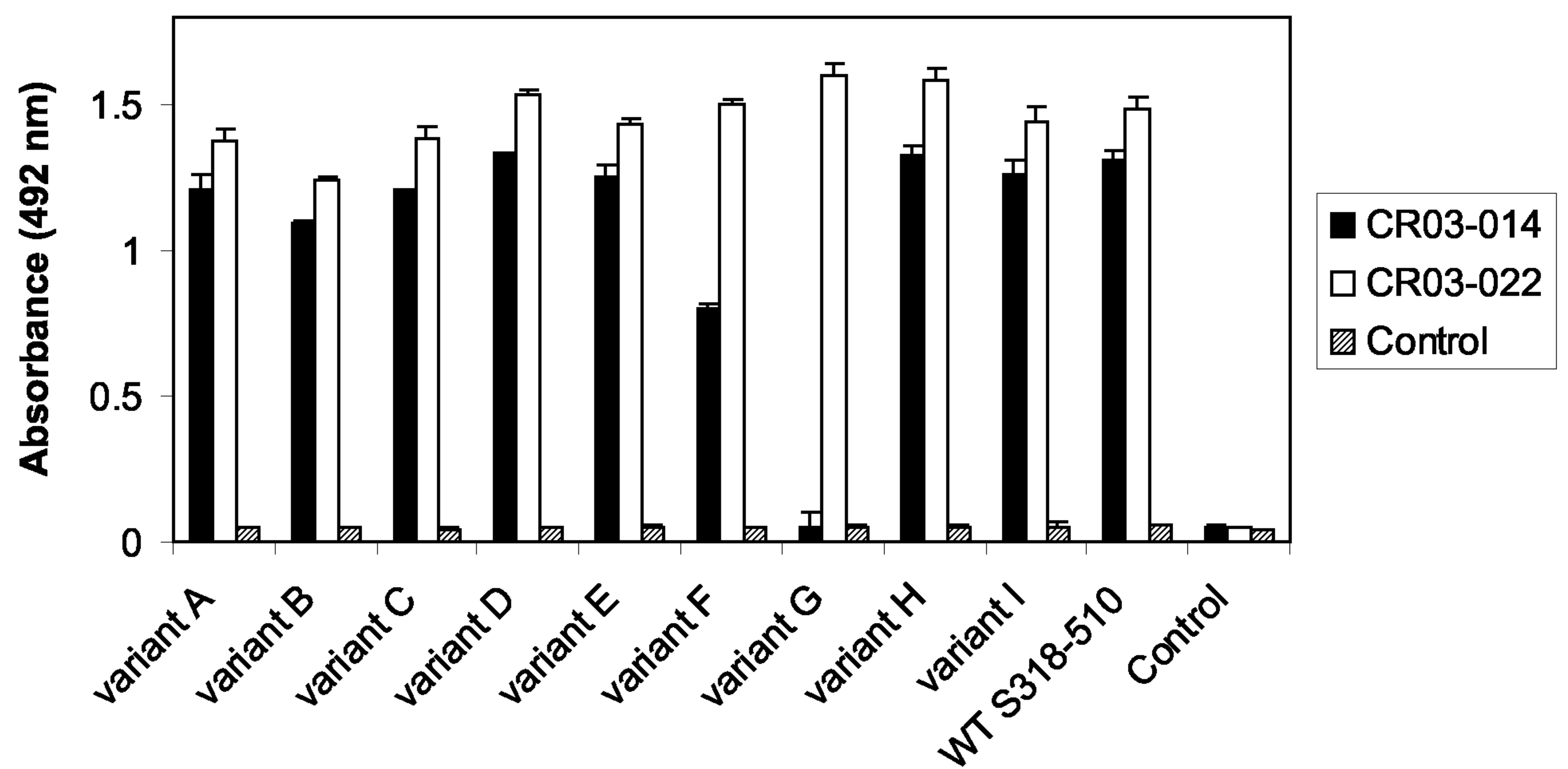


FIGURE 8

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				462			
	P	F	S	P	D	G	K
SARS wt	1375-CCT	TTC	TCC	CCT	GAT	GGC	AAA
	P	F	S	L	D	G	K
E014-C06	1375-CCT	TTC	TCC	C TT	GAT	GGC	AAA
E014-C07	1375-CCT	TTC	TCC	C TT	GAT	GGC	AAA
E014-C08	1375-CCT	TTC	TCC	C TT	GAT	GGC	AAA
E014-C09	1375-CCT	TTC	TCC	C TT	GAT	GGC	AAA
E014-C10	1375-CCT	TTC	TCC	C TT	GAT	GGC	AAA

FIGURE 9

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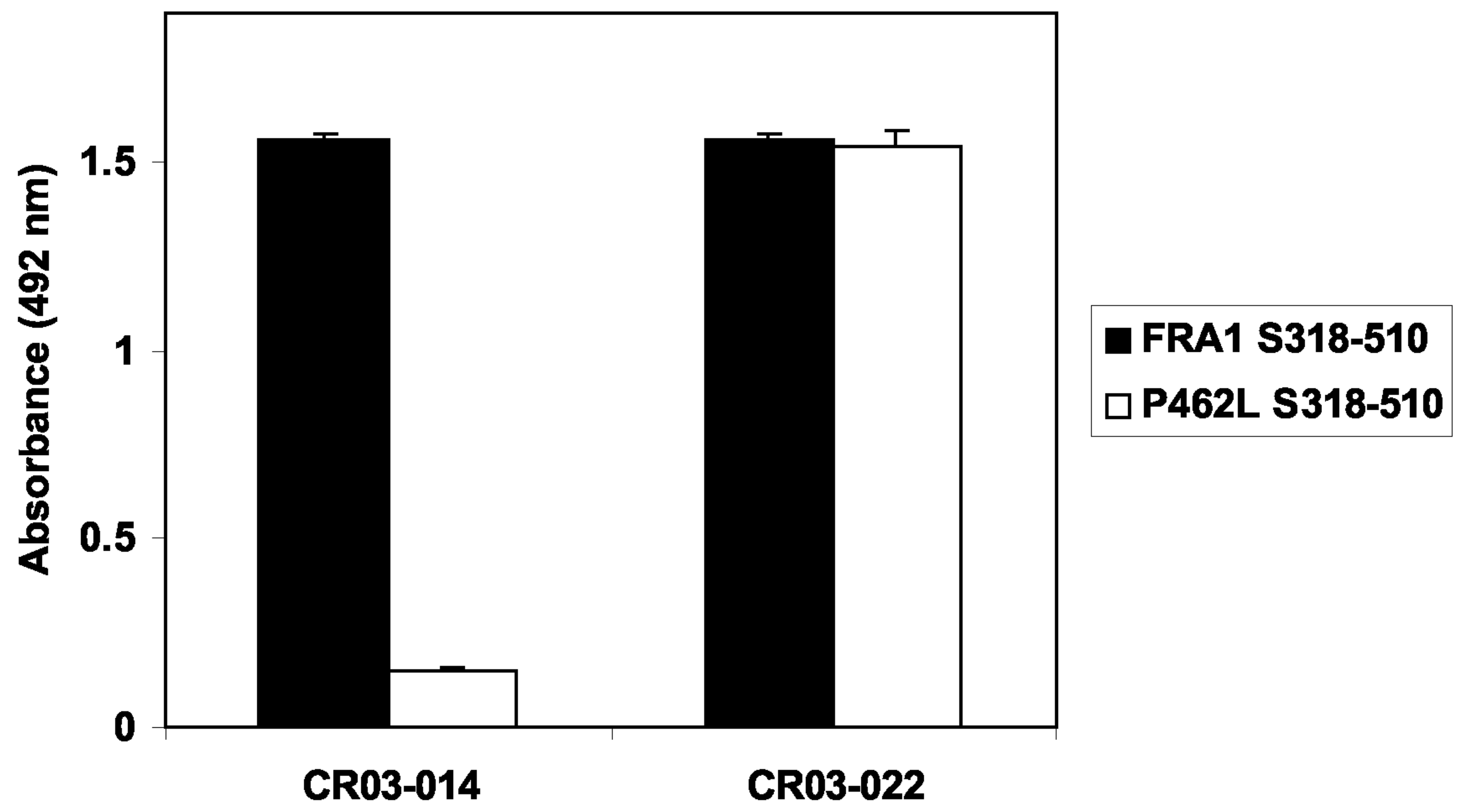


FIGURE 10