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(54) **Title**: PEPTIDE REAGENTS AND METHOD FOR INHIBITING AUTOANTIBODY ANTIGEN BINDING

Ala Asp Gly Ser Ser Asp Ala Ala Arg Glu Pro Arg Pro Ala Pro Ala Pro Ile Arg Arg Arg Ser Ser Asn Tyr Arg Ala Tyr Ala Thr Glu Pro His Ala Lys Lys Lys Ser Lys Ile Ser Ala Ser Arg Lys Leu Gln Leu Lys Thr Leu Leu Leu Gln Ile Ala Lys Gln Glu Leu Glu Arg Glu Ala Glu Glu Arg Arg Gly Glu Lys Gly Arg Ala Leu Ser Thr Arg Cys Gln Pro Leu Glu Leu Ala Gly Leu Gly Phe Ala Glu Leu Gln Asp Leu Cys Arg Gln Leu His Ala Arg Val Asp Lys Val Asp Glu Glu Arg Tyr Asp Ile Glu Ala Lys Val Thr Lys Asn Ile Thr Glu Ile Ala Asp Leu Thr Gln Lys Ile Phe Asp Leu Arg Gly Lys Phe Lys Arg Pro Thr Leu Arg Arg Val Arg Ile Ser Ala Asp Ala Met Met Gln Ala Leu Leu Gly Ala Arg Ala Lys Glu Ser Leu Asp Leu Arg Ala His Leu Lys Gln Val Lys Lys Glu Asp Thr Glu Lys Glu Asn Arg Glu Val Gly Asp Trp Arg Lys Asn Ile Asp Ala Leu Ser Gly Met Glu Gly Arg Lys Lys Lys Phe Glu

FIG. 1

(57) **Abstract**: The present disclosure provides immunoassays and kits for detection or quantification of an protein of interest in a test sample that potentially contains endogenously produced autoantibodies reactive with the analyte.

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PEPTIDE REAGENTS AND METHOD FOR INHIBITING AUTOANTIBODY ANTIGEN BINDING

RELATED APPLICATION INFORMATION

None.

INCORPORATION OF SEQUENCE LISTING

The entire contents of a paper copy of the "Sequence Listing" and a computer readable form of the sequence listing on diskette, containing the file named 400797_SequenceListing_ST25.txt, which is 56 kilobytes in size and was created on November 17, 2010, 2009, are herein incorporated by reference.

TECHNICAL FIELD

The present disclosure relates to methods and kits for detecting a protein of interest in a test sample, and in particular to methods and kits for detecting the protein in a human test sample that may contain endogenous anti-analyte antibodies.

BACKGROUND

Immunoassay techniques have been known for the last few decades and are now commonly used in medicine for a wide variety of diagnostic purposes to detect target analytes in a biological sample. Immunoassays exploit the highly specific binding of an antibody to its corresponding antigen, wherein the antigen is the target analyte. Typically, quantification of either the antibody or antigen is achieved through some form of labeling such as radio- or fluorescence-labeling. Sandwich immunoassays involve binding the target analyte in the sample to the antibody site (which is frequently bound to a solid support), binding labeled antibody to the captured analyte, and then measuring the amount of bound labeled antibody, wherein the label generates a signal proportional to the concentration of the target analyte inasmuch as labeled antibody does not bind unless the analyte is present in the sample.

A problem with this general approach is that many patients have circulating endogenous antibodies, or "autoantibodies" against an analyte of clinical interest. For example, autoantibodies have been described for cardiac troponin, myeloperoxidase (MPO), prostate specific antigen (PSA), and thyroid stimulating hormone (TSH), and other clinically significant analytes. Autoantibodies create interference in typical sandwich immunoassays that are composed of two or more analyte-specific antibodies. For example, cardiac troponin-reactive autoantibodies may interfere with the measurement of cTnI using conventional midfragment-specific immunoassays. Thus, interference from autoantibodies can produce

erroneous results, particularly near the cut-off values established for clinical diagnoses, and increases the risk of false negative diagnostic results and the risk that individuals will not obtain a timely diagnosis.

One approach to addressing this problem is to choose analyte-specific antibodies that bind to specific epitopes distinct from the analyte epitopes that react with the autoantibodies. Following this general approach, efforts have focused on exploring the use of thousands of different combinations of two, three and even four analyte-specific antibodies to avoid interference from autoantibodies. However, this effort has been largely unsuccessful. It is now evident that autoantibodies against complex protein analytes are likely to be polyclonal within a particular sample, and may be even more diverse among samples from different individuals. Interference from diverse polyclonal autoantibodies may explain the observation that as little as 25% or even less of an analyte protein sequence binds to analyte-specific antibodies, which may in turn explain the lack of success using this approach.

A need exists in the art for new immunoassay methods that compensate for interference by autoantibodies in a sample, and in particular for such methods that do so without involving redesign of the analyte detection or capture antibodies.

SUMMARY

In one embodiment, the present disclosure relates to a reagent for use in an immunoassay for determining the presence or amount of at least one protein in a test sample, the reagent comprising at least one peptide comprising at least 5 consecutive amino acid residues wherein the peptide is derived from the protein and further wherein the reagent is used to block the interaction between an endogenous antibody and the protein in the test sample.

In certain embodiments, the protein from which the reagent is derived may be selected from the group consisting of: cardiac troponin I (SEQ ID NO:1), cardiac troponin T (SEQ ID NO:2), thyroid stimulating hormone (TSH) (SEQ ID NO:3), beta-human chorionic gonadotropin (beta-HCG) (SEQ ID NO:4), myeloperoxidase (MPO) (SEQ ID NO:5), prostate specific antigen (PSA) (SEQ ID NO:6), human B-type natriuretic peptide (hBNP) (SEQ ID NO:7), myosin light chain 2 (SEQ ID NO:8), myosin-6 (SEQ ID NO:9) and myosin-7 (SEQ ID NO:10).

The peptide can have, for example, an amino acid sequence of five (5) consecutive amino acid residues to fifteen (15) consecutive amino acid residues from the amino acid sequence of the protein from which the reagent is derived. In one embodiment, for example,

the protein from which the reagent is derived is cardiac troponin I, and the reagent has an amino acid sequence comprising at least five consecutive amino acid residues from the full amino acid sequence of cardiac troponin I (SEQ ID NO: 1). In certain embodiments, the peptide reagent has a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15), or any subsequence thereof consisting of at least 5 consecutive amino acid residues.

In another embodiment, the present disclosure relates to a reagent for use in an immunoassay for determining the presence or amount of a cardiac troponin I in a test sample, the reagent comprising a peptide having a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

In another embodiment, the present disclosure relates to a method of detecting at least one protein of interest in a test sample, the method comprising the steps of:

a. preparing a first mixture comprising a test sample suspected of containing at least one protein of interest and at least one reagent, wherein said reagent (1) is at least one peptide comprising at least 5 consecutive amino acid residues derived from said protein that binds to the antibody of interest; and (2) disrupts the interaction between an endogenous antibody in the test sample and the antigen;

b. preparing a second mixture comprising the first mixture and a first specific binding partner, wherein the first specific binding partner comprises an antibody, wherein the antibody binds with the protein of interest to form a first specific binding partner-protein complex; and

c. contacting the second mixture with a second specific binding partner, wherein the second specific binding partner comprises an antibody that has been conjugated to a detectable label and further wherein the second specific binding partner binds to the first specific binding partner-protein complex to form a first specific binding partner-protein-second specific binding partner complex; and

d. measuring the signal generated by or emitted from the detectable label and detecting the protein of interest in the test sample.

In the above-described method, the protein can be selected for example from the group consisting of: cardiac troponin I, cardiac troponin T, thyroid stimulating hormone

(TSH), beta-human chorionic gonadotropin (beta-HCG), myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (hBNP), myosin light chain 2, myosin-6 and myosin-

In the above-described method the test sample can be whole blood, serum or plasma.

In one embodiment of the method, the first specific binding partner can be immobilized to a solid phase either before or after the formation of the first specific binding partner-protein complex. Additionally, the second specific binding partner can be immobilized to a solid phase either before or after formation of the first specific binding partner-protein-second specific binding partner complex.

In the above-described method the detectable label can be selected from the group consisting of a radioactive label, an enzymatic label, a chemiluminescent label, a fluorescence label, a thermometric label, and an immuno-polymerase chain reaction label.

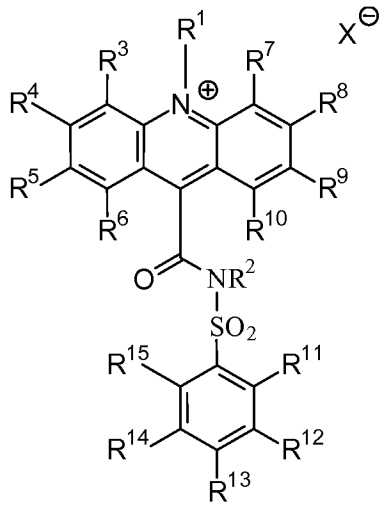
In one embodiment of the method the detectable label is an acridinium compound. When an acridinium compound is used, the method may further include:

a. generating or providing a source of hydrogen peroxide to the second mixture contacted with a second specific binding partner;

b. adding a basic solution to the mixture of step (a); and

c. measuring the light signal generated or emitted in step (b) and detecting the protein of interest in the sample.

Any acridinium compound can be used in the above-described method. For example, the acridinium compound can be an acridinium-9-carboxamide having a structure according to formula I:

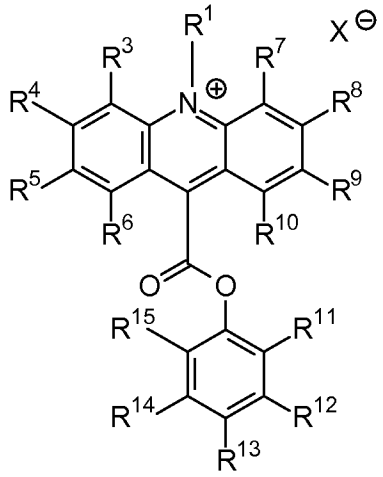


I

wherein R1 and R2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and optionally, if present, XΘ is an anion.

Alternatively, the acridinium compound can be an acridinium-9-carboxylate aryl ester having a structure according to formula II:



II

wherein R1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X\Theta$ is an anion.

In the above-described method, the reagent can be a peptide having a length of 5 consecutive amino acids to 15 consecutive amino acids.

In one embodiment of the method, the protein from which the peptide is derived is cardiac troponin I, and the peptide has a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

The above-described method may further include the step of quantifying the amount of protein of interest in the test sample by relating the amount of signal in step (c) to the amount of the one or more proteins of interest in the test sample either by use of a standard curve for the protein of interest or by comparison to a reference standard.

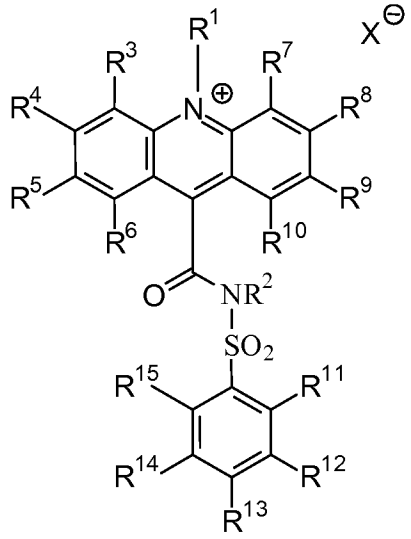
The above-described method may be adapted for use in an automated system or semi-automated system.

In still another embodiment, the present disclosure relates to a kit for detecting and/or quantifying at least one protein of interest in a test sample, the kit comprising the above-described peptide reagent, a capture reagent comprising an antibody that binds to the protein of interest, and instructions for detecting and/or quantifying at least one protein of interest in a test sample.

The above-described kit may further include a conjugate comprising an antibody conjugated to a detectable label.

In one embodiment of the kit, the detectable label can be selected from the group consisting of a radioactive label, an enzymatic label, a chemiluminescent label, a fluorescence label, a thermometric label, and an immuno-polymerase chain reaction label.

The detectable label used in the above-described kit can be an acridinium compound. Any acridinium compound can be used. For example the acridinium compound can be an acridinium-9-carboxamide having a structure according to formula I:



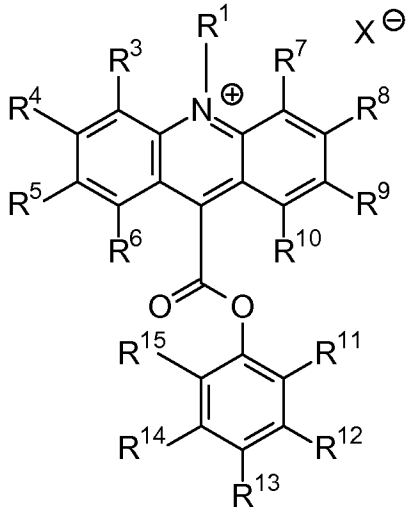
I

wherein R1 and R2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion.

Alternatively, the acridinium compound can be an acridinium-9-carboxylate aryl ester having a structure according to formula II:



II

wherein R1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, XΘ is an anion..

When an acridinium compound is included as the detectable label in the above-described kit, the kit optionally further includes a basic solution. The basic solution can be for example a solution having a pH of at least about 10.

The above kit may further include a hydrogen peroxide source, which can be a buffer, a solution containing hydrogen peroxide, or a hydrogen peroxide generating enzyme. In kits containing a hydrogen peroxide generating enzyme, the enzyme can be selected from the group consisting of: (R)-6-hydroxynicotine oxidase, (S)-2-hydroxy acid oxidase, (S)-6-hydroxynicotine oxidase, 3-aci-nitropropanoate oxidase, 3-hydroxyanthranilate oxidase, 4-hydroxymandelate oxidase, 6-hydroxynicotinate dehydrogenase, abscisic-aldehyde oxidase, acyl-CoA oxidase, alcohol oxidase, aldehyde oxidase, amine oxidase, amine oxidase (copper-containing), amine oxidase (flavin-containing), aryl-alcohol oxidase, aryl-aldehyde oxidase, catechol oxidase, cholesterol oxidase, choline oxidase, columbamine oxidase, cyclohexylamine oxidase , cytochrome c oxidase, D-amino-acid oxidase, D-arabinono-1,4-lactone oxidase, D-arabinono-1,4-lactone oxidase, D-aspartate oxidase, D-glutamate oxidase, D-glutamate(D-aspartate) oxidase, dihydrobenzophenanthridine oxidase, dihydroorotate oxidase, dihydrouracil oxidase, dimethylglycine oxidase, D-mannitol oxidase, ecdysone oxidase, ethanolamine oxidase, galactose oxidase , glucose oxidase , glutathione oxidase, glycerol-3-phosphate oxidase, glycine oxidase, glyoxylate oxidase, hexose oxidase, hydroxyphytanate oxidase, indole-3-acetaldehyde oxidase, lactic acid oxidase, L-amino-acid oxidase, L-aspartate oxidase, L-galactonolactone oxidase, L-glutamate oxidase, L-gulonolactone oxidase, L-lysine 6-oxidase, L-lysine oxidase, long-chain-alcohol oxidase, L-pipecolate oxidase, L-sorbose oxidase, malate oxidase, methanethiol oxidase, monoamino acid oxidase , N6-methyl-lysine oxidase, N-acylhexosamine oxidase, NAD(P)H oxidase, nitroalkane oxidase, N-methyl-L-amino-acid oxidase, nucleoside oxidase, oxalate oxidase, polyamine oxidase, polyphenol oxidase, polyvinyl-alcohol oxidase, prenylcysteine oxidase, protein-lysine 6-oxidase, putrescine oxidase, pyranose oxidase, pyridoxal 5′-phosphate synthase, pyridoxine 4-oxidase, pyrroloquinoline-quinone synthase, pyruvate oxidase, pyruvate oxidase (CoA-acetylating), reticuline oxidase, retinal oxidase, rifamycin-B oxidase,

sarcosine oxidase, secondary-alcohol oxidase, sulfite oxidase, superoxide dismutase, superoxide reductase, tetrahydroberberine oxidase, thiamine oxidase, tryptophan α,β-oxidase, urate oxidase (uricase, uric acid oxidase), vanillyl-alcohol oxidase, xanthine oxidase, xylitol oxidase and combinations thereof.

In one embodiment, the above-described kit includes a reagent derived from a protein selected from the group consisting of: cardiac troponin I, cardiac troponin T, thyroid stimulating hormone (TSH), beta-human chorionic gonadotropin (beta-HCG), myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (hBNP), myosin light chain 2, myosin-6 and myosin-7.

In the above-described kit, the reagent can be a peptide having a length of 5 consecutive amino acids to 15 consecutive amino acids.

In one embodiment of the above-described kit, the protein from which the reagent is derived is cardiac troponin I, and the peptide has a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the amino acid sequence of cardiac troponin I;

Figure 2 shows the amino acid sequence of cardiac troponin T;

Figure 3 shows the amino acid sequence of thyroid stimulating hormone (TSH);

Figure 4 shows the amino acid sequence of the beta subunit of human chorionic gonadotropin (beta-HCG);

Figure 5 shows the amino acid sequence of myeloperoxidase (MPO);

Figure 6 shows the amino acid sequence of prostate specific antigen (PSA);

Figure 7 shows the amino acid sequence of human B-type natriuretic peptide (hBNP);

Figure 8 shows the amino acid sequence of myosin light chain 2;

Figure 9 A-C shows the amino acid sequence of myosin-6;

Figure 10 A-C shows the amino acid sequence of myosin-7;

Figure 11 shows a graph of the ratio of the signal to the low control (S/LC) against concentration (nmol/mL) for each of five different peptide reagents and a combination thereof; and

Figure 12 shows a graph of the ratio of the signal to the low control (S/LC) against concentration (nmol/mL) for each of five different peptide reagents and a combination thereof.

DETAILED DESCRIPTION

The present disclosure relates to immunoassay methods and kits for detecting a protein of interest in a test sample, and more particularly to methods and kits for detecting a protein in a human test sample that may contain endogenous antibodies against the protein of interest. Specifically, the inventors have discovered an alternative approach to address the problem of interference by autoantibodies in immunodetection of clinically significant analytes in a sample. Such analytes include self-antigens such as for example cardiac troponin, myeloperoxidase, prostate specific antigen and thyroid stimulating hormone. More specifically, the alternative approach includes use of a peptide reagent that is derived from the protein, especially a self-antigen, of interest. The peptide reagent inhibits binding of autoantibodies to the protein, and thus prevents interference by autoantibodies with immunodetection of the protein. This approach compensates for the presence of autoantibodies that may be in the sample without need for a redesign of the specific detection antibodies or the capture antibodies, does not require use of an extra anti-human IgG detection conjugate, and avoids the need of a second assay to identify problematic samples.

A. Definitions

Section headings as used in this section and the entire disclosure herein are not intended to be limiting.

As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9 and 7.0 are explicitly contemplated.

a) Acyl (and other chemical structural group definitions)

As used herein, the term "acyl" refers to a $-C(O)R_a$ group where R_a is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl. Representative examples of acyl include, but are not limited to, formyl, acetyl, cylcohexylcarbonyl, cyclohexylmethylcarbonyl, benzoyl, benzylcarbonyl and the like.

As used herein, the term "alkenyl" means a straight or branched chain hydrocarbon containing from 2 to 10 carbons and containing at least one carbon-carbon double bond formed by the removal of two hydrogens. Representative examples of alkenyl include, but are not limited to, ethenyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, 5-hexenyl, 2-heptenyl, 2-methyl-1-heptenyl, and 3-decenyl.

As used herein, the term "alkyl" means a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, and n-decyl.

As used herein, the term "alkyl radical" means any of a series of univalent groups of the general formula C_nH_{2n+1} derived from straight or branched chain hydrocarbons.

As used herein, the term "alkoxy" means an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, tert-butoxy, pentyloxy, and hexyloxy.

As used herein, the term "alkynyl" means a straight or branched chain hydrocarbon group containing from 2 to 10 carbon atoms and containing at least one carbon-carbon triple bond. Representative examples of alkynyl include, but are not limited, to acetylenyl, 1-propynyl, 2-propynyl, 3-butynyl, 2-pentynyl, and 1-butynyl.

As used herein, the term "amido" refers to an amino group attached to the parent molecular moiety through a carbonyl group (wherein the term "carbonyl group" refers to a –C(O)– group).

As used herein, the term "amino" means $–NR_bR_c$, wherein R_b and R_c are independently selected from the group consisting of hydrogen, alkyl and alkylcarbonyl.

As used herein, the term "aralkyl" means an aryl group appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of arylalkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, and 2-naphth-2-ylethyl.

As used herein, the term "aryl" means a phenyl group, or a bicyclic or tricyclic fused ring system wherein one or more of the fused rings is a phenyl group. Bicyclic fused ring systems are exemplified by a phenyl group fused to a cycloalkenyl group, a cycloalkyl group, or another phenyl group. Tricyclic fused ring systems are exemplified by a bicyclic fused ring system fused to a cycloalkenyl group, a cycloalkyl group, as defined herein or another

phenyl group. Representative examples of aryl include, but are not limited to, anthracenyl, azulenyl, fluorenyl, indanyl, indenyl, naphthyl, phenyl, and tetrahydronaphthyl. The aryl groups of the present disclosure can be optionally substituted with one-, two, three, four, or five substituents independently selected from the group consisting of alkoxy, alkyl, carboxyl, halo, and hydroxyl.

As used herein, the term "carboxy" or "carboxyl" refers to $-CO_2H$ or $-CO_2^-$.

As used herein, the term "carboxyalkyl" refers to a $-(CH_2)_nCO_2H$ or $-(CH_2)_nCO_2^-$ group where n is from 1 to 10.

As used herein, the term "cyano" means a –CN group.

As used herein, the term "cycloalkenyl" refers to a non-aromatic cyclic or bicyclic ring system having from three to ten carbon atoms and one to three rings, wherein each five-membered ring has one double bond, each six-membered ring has one or two double bonds, each seven- and eight-membered ring has one to three double bonds, and each nine-to ten-membered ring has one to four double bonds. Representative examples of cycloalkenyl groups include cyclohexenyl, octahydronaphthalenyl, norbornylenyl, and the like. The cycloalkenyl groups can be optionally substituted with one, two, three, four, or five substituents independently selected from the group consisting of alkoxy, alkyl, carboxyl, halo, and hydroxyl.

As used herein, the term "cycloalkyl" refers to a saturated monocyclic, bicyclic, or tricyclic hydrocarbon ring system having three to twelve carbon atoms. Representative examples of cycloalkyl groups include cyclopropyl, cyclopentyl, bicyclo[3.1.1]heptyl, adamantyl, and the like. The cycloalkyl groups of the present disclosure can be optionally substituted with one, two, three, four, or five substituents independently selected from the group consisting of alkoxy, alkyl, carboxyl, halo, and hydroxyl.

As used herein, the term "cycloalkylalkyl" means a $-R_dR_e$ group where R_d is an alkylene group and R_e is cycloalkyl group. A representative example of a cycloalkylalkyl group is cyclohexylmethyl and the like.

As used herein, the term "halogen" means a –Cl, –Br, –I or –F; the term "halide" means a binary compound, of which one part is a halogen atom and the other part is an element or radical that is less electronegative than the halogen, e.g., an alkyl radical.

As used herein, the term "hydroxyl" means an –OH group.

As used herein, the term "nitro" means a $-NO_2$ group.

As used herein, the term "oxoalkyl" refers to $-(CH_2)_nC(O)R_a$, where R_a is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl and where n is from 1 to 10.

As used herein, the term “phenylalkyl” means an alkyl group which is substituted by a phenyl group.

As used herein, the term “sulfo” means a $–SO_3H$ group.

As used herein, the term “sulfoalkyl” refers to a $–(CH_2)_nSO_3H$ or $–(CH_2)_nSO_3^-$ group where n is from 1 to 10.

b) Anion

As used herein, the term “anion” refers to an anion of an inorganic or organic acid, such as, but not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, methane sulfonic acid, formic acid, acetic acid, oxalic acid, succinic acid, tartaric acid, mandelic acid, fumaric acid, lactic acid, citric acid, glutamic acid, aspartic acid, phosphate, trifluoromethansulfonic acid, trifluoroacetic acid and fluorosulfonic acid and any combinations thereof.

c) Antibody

As used herein, the term "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes, and encompasses polyclonal antibodies, monoclonal antibodies, and fragments thereof, as well as molecules engineered from immunoglobulin gene sequences. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

d) Hydrogen Peroxide Generating Enzyme

As used herein, the term “hydrogen peroxide generating enzyme” refers to an enzyme that is capable of producing as a reaction product the chemical compound having the molecular formula H_2O_2, i.e. hydrogen peroxide. Non-limiting examples of hydrogen peroxide generating enzymes are listed below in Table 1.

Table 1

ACCEPTED COMMON NAME	IUBMB ENZYME NOMENCLATURE	PREFERRED SUBSTRATE
(R)-6-hydroxynicotine oxidase	EC 1.5.3.6	(R)-6-hydroxynicotine
(S)-2-hydroxy acid oxidase	EC 1.1.3.15	S)-2-hydroxy acid
(S)-6-hydroxynicotine oxidase	EC 1.5.3.5	(S)-6-hydroxynicotine

3-aci-nitropropanoate oxidase	EC 1.7.3.5	3-aci-nitropropanoate
3-hydroxyanthranilate oxidase	EC 1.10.3.5	3-hydroxyanthranilate
4-hydroxymandelate oxidase	EC 1.1.3.19	(S)-2-hydroxy-2-(4-hydroxyphenyl)acetate
6-hydroxynicotinate dehydrogenase	EC 1.17.3.3	6-hydroxynicotinate
Abscisic-aldehyde oxidase	EC 1.2.3.14	abscisic aldehyde
acyl-CoA oxidase	EC 1.3.3.6	acyl-CoA
Alcohol oxidase	EC 1.1.3.13	a primary alcohol
Aldehyde oxidase	EC 1.2.3.1	an aldehyde
amine oxidase		
amine oxidase (copper-containing)	EC 1.4.3.6	primary monoamines, diamines and histamine
amine oxidase (flavin-containing)	EC 1.4.3.4	a primary amine
aryl-alcohol oxidase	EC 1.1.3.7	an aromatic primary alcohol (2-naphthyl)methanol 3-methoxybenzyl alcohol
aryl-aldehyde oxidase	EC 1.2.3.9	an aromatic aldehyde
Catechol oxidase	EC 1.1.3.14	Catechol
cholesterol oxidase	EC 1.1.3.6	Cholesterol
Choline oxidase	EC 1.1.3.17	Choline
columbamine oxidase	EC 1.21.3.2	Columbamine
cyclohexylamine oxidase	EC 1.4.3.12	Cyclohexylamine
cytochrome c oxidase	EC 1.9.3.1	
D-amino-acid oxidase	EC 1.4.3.3	a D-amino acid
D-arabinono-1,4-lactone oxidase	EC 1.1.3.37	D-arabinono-1,4-lactone
D-arabinono-1,4-lactone oxidase	EC 1.1.3.37	D-arabinono-1,4-lactone
D-aspartate oxidase	EC 1.4.3.1	D-aspartate
D-glutamate oxidase	EC 1.4.3.7	D-glutamate
D-glutamate(D-aspartate) oxidase	EC 1.4.3.15	D-glutamate
dihydrobenzophenanthridine oxidase	EC 1.5.3.12	dihydrosanguinarine

dihydroorotate oxidase	EC 1.3.3.1	(S)-dihydroorotate
dihydrouracil oxidase	EC 1.3.3.7	5,6-dihydrouracil
dimethylglycine oxidase	EC 1.5.3.10	N,N-dimethylglycine
D-mannitol oxidase	EC 1.1.3.40	Mannitol
Ecdysone oxidase	EC 1.1.3.16	Ecdysone
ethanolamine oxidase	EC 1.4.3.8	Ethanolamine
Galactose oxidase	EC 1.1.3.9	D-galactose
Glucose oxidase	EC 1.1.3.4	β-D-glucose
glutathione oxidase	EC 1.8.3.3	Glutathione
Glycerol-3-phosphate oxidase	EC 1.1.3.21	sn-glycerol 3-phosphate
Glycine oxidase	EC 1.4.3.19	Glycine
glyoxylate oxidase	EC 1.2.3.5	Glyoxylate
hexose oxidase	EC 1.1.3.5	D-glucose, D-galactose D-mannose maltose lactose cellobiose
hydroxyphytanate oxidase	EC 1.1.3.27	L-2-hydroxyphytanate
indole-3-acetaldehyde oxidase	EC 1.2.3.7	(indol-3-yl)acetaldehyde
lactic acid oxidase		Lactic acid
L-amino-acid oxidase	EC 1.4.3.2	an L-amino acid
L-aspartate oxidase	EC 1.4.3.16	L-aspartate
L-galactonolactone oxidase	EC 1.3.3.12	L-galactono-1,4-lactone
L-glutamate oxidase	EC 1.4.3.11	L-glutamate
L-gulonolactone oxidase	EC 1.1.3.8	L-gulono-1,4-lactone
L-lysine 6-oxidase	EC 1.4.3.20	L-lysine
L-lysine oxidase	EC 1.4.3.14	L-lysine
long-chain-alcohol oxidase	EC 1.1.3.20	A long-chain-alcohol
L-pipecolate oxidase	EC 1.5.3.7	L-pipecolate
L-sorbose oxidase	EC 1.1.3.11	L-sorbose
malate oxidase	EC 1.1.3.3	(S)-malate
methanethiol oxidase	EC 1.8.3.4	Methanethiol

monoamino acid oxidase		
N^6-methyl-lysine oxidase	EC 1.5.3.4	6-N-methyl-L-lysine
N-acylhexosamine oxidase	EC 1.1.3.29	N-acetyl-D-glucosamine N-glycolylglucosamine N-acetylgalactosamine N-acetylmannosamine.
NAD(P)H oxidase	EC 1.6.3.1	NAD(P)H
nitroalkane oxidase	EC 1.7.3.1	a nitroalkane
N-methyl-L-amino-acid oxidase	EC 1.5.3.2	an N-methyl-L-amino acid
nucleoside oxidase	EC 1.1.3.39	Adenosine
Oxalate oxidase	EC 1.2.3.4	Oxalate
polyamine oxidase	EC 1.5.3.11	1-N-acetylspermine
polyphenol oxidase	EC 1.14.18.1	
Polyvinyl-alcohol oxidase	EC 1.1.3.30	polyvinyl alcohol
prenylcysteine oxidase	EC 1.8.3.5	an S-prenyl-L-cysteine
Protein-lysine 6-oxidase	EC 1.4.3.13	peptidyl-L-lysyl-peptide
putrescine oxidase	EC 1.4.3.10	butane-1,4-diamine
Pyranose oxidase	EC 1.1.3.10	D-glucose D-xylose L-sorbose D-glucono-1,5-lactone
Pyridoxal 5′-phosphate synthase	EC 1.4.3.5	pyridoxamine 5′-phosphate
pyridoxine 4-oxidase	EC 1.1.3.12	Pyridoxine
pyrroloquinoline-quinone synthase	EC 1.3.3.11	6-(2-amino-2-carboxyethyl)-7,8-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-2,4-dicarboxylate
Pyruvate oxidase	EC 1.2.3.3	Pyruvate
Pyruvate oxidase (CoA-acetylating)	EC 1.2.3.6	Pyruvate
Reticuline oxidase	EC 1.21.3.3	Reticuline

retinal oxidase	EC 1.2.3.11	Retinal
Rifamycin-B oxidase	EC 1.10.3.6	rifamycin-B
Sarcosine oxidase	EC 1.5.3.1	Sarcosine
secondary-alcohol oxidase	EC 1.1.3.18	a secondary alcohol
sulfite oxidase	EC 1.8.3.1	Sulfite
superoxide dismutase	EC 1.15.1.1	Superoxide
superoxide reductase	EC 1.15.1.2	Superoxide
tetrahydroberberine oxidase	EC 1.3.3.8	(S)-tetrahydroberberine
Thiamine oxidase	EC 1.1.3.23	Thiamine
tryptophan α,β-oxidase	EC 1.3.3.10	L-tryptophan
urate oxidase (uricase, uric acid oxidase)	EC 1.7.3.3	uric acid
Vanillyl-alcohol oxidase	EC 1.1.3.38	vanillyl alcohol
Xanthine oxidase	EC 1.17.3.2	Xanthine
xylitol oxidase	EC 1.1.3.41	Xylitol

e) Autoantibody

As used herein, the phrase "autoantibody" refers to an antibody that binds to an analyte that is endogenously produced in the subject in which the antibody is produced.

f) Specific Binding Partner

As used herein, the phrase "specific binding partner," as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules, through chemical or physical means, specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors, and enzymes and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal and complexes thereof, including those formed by recombinant DNA molecules.

g) Specific Binding Partner-Protein Complex

As used herein, the phrase "specific binding partner-protein complex" refers to a combination of an antibody and an antigen, in which the antigen is a protein of interest, and the antibody and protein are bound by specific, noncovalent interactions between an antigen-combining site on the antibody and an antigen epitope.

h) Detectable Label

As used herein the term "detectable label" refers to any moiety that generates a measurable signal via optical, electrical, or other physical indication of a change of state of a molecule or molecules coupled to the moiety. Such physical indicators encompass spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, radiochemical, and chemical means, such as but not limited to fluorescence, chemifluorescence, chemiluminescence, and the like. Preferred detectable labels include acridinium compounds such as an acridinium-9-carboximide having a structure according to Formula I as set forth in section B herein below, and an acridinium-9-carboxylate aryl ester having a structure according to Formula II as also set forth in section B herein below.

i) Subject

As used herein, the terms "subject" and "patient" are used interchangeably irrespective of whether the subject has or is currently undergoing any form of treatment. As used herein, the terms "subject" and "subjects" refer to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous monkey, chimpanzee, etc) and a human). Preferably, the subject is a human.

j) Test Sample

As used herein, the term "test sample" generally refers to a biological material being tested for and/or suspected of containing an protein of interest and which may also include autoantibodies to the protein of interest. The biological material may be derived from any biological source but preferably is a biological fluid likely to contain the protein of interest. Examples of biological materials include, but are not limited to, stool, whole blood, serum, plasma, red blood cells, platelets, interstitial fluid, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, ascites fluid, mucous, nasal fluid, sputum, synovial fluid, peritoneal fluid, vaginal fluid, menses, amniotic fluid, semen, soil, etc. The test sample may be used directly as obtained from the biological source or following a pretreatment to modify the character of the sample. For example, such pretreatment may include preparing plasma from blood, diluting viscous fluids and so forth. Methods of pretreatment may also involve filtration, precipitation, dilution, distillation, mixing, concentration, inactivation of interfering

components, the addition of reagents, lysing, etc. If such methods of pretreatment are employed with respect to the test sample, such pretreatment methods are such that the protein of interest remains in the test sample at a concentration proportional to that in an untreated test sample (e.g., namely, a test sample that is not subjected to any such pretreatment method(s)).

B. Peptide Reagents

Self-antigens include a number of proteins that are known to be endogenously produced in relation to a particular disease state or injury in a subject. Self-antigens for which autoantibodies have been identified include the troponins, namely cardiac troponin I (SEQ ID NO:1), and cardiac troponin T (SEQ ID NO:2); thyroid stimulating hormone (TSH) (SEQ ID NO:3); the beta subunit of human chorionic gonadotropin (beta-HCG) (SEQ ID NO:4); myeloperoxidase (MPO) (SEQ ID NO:5); prostate specific antigen (PSA) (SEQ ID NO:6); human B-type natriuretic peptide (hBNP) (SEQ ID NO:7); myosin light chain 2 (SEQ ID NO:8); myosin-6 (SEQ ID NO:9) and myosin-7 (SEQ ID NO:10).

The peptide reagents of the present disclosure are derived from the amino acid sequence of the target self-antigen, and can be used in an immunoassay format to prevent interference by autoantibodies against the self-antigen. More specifically, the peptide reagent is used to block the interaction between the self-antigen and any autoantibodies against the self-antigen that may be present in a test sample. Each peptide reagent may be used alone, or in combination with one or more other peptide reagents derived from the target protein. A synergistic blocking effect is believed to result from a combination of different peptide reagents derived from the same target protein.

The peptide reagent includes at least five (5) consecutive amino acid residues from the amino acid sequence of the target self-antigen. In one embodiment, the peptide reagent includes five (5) to fifteen (15) consecutive amino acid residues from the amino acid sequence of the target self-antigen. For example, given cardiac troponin I as the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of cardiac troponin I (Figure 1; SEQ ID NO: 1). For example, the peptide reagent can comprise any of the following amino acid sequences: ADGSS (residues 1-5), KFFES (residues 205-209), or KKKSKISASRKLQLK (residues 35-49), or any other sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of cardiac troponin I (SEQ ID NO: 1). Table 2 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid

residues from cardiac troponin I (SEQ ID NO: 1). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 2, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:1, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 2:

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

	Ala Tyr Ala Thr Glu		Thr Arg Cys Gln Pro		Leu Thr Gln Lys Ile
	Tyr Ala Thr Glu Pro		Arg Cys Gln Pro Leu		Thr Gln Lys Ile Phe
	Ala Thr Glu Pro His		Cys Gln Pro Leu Glu		Gln Lys Ile Phe Asp
	Thr Glu Pro His Ala		Gln Pro Leu Glu Leu		Lys Ile Phe Asp Leu
	Glu Pro His Ala Lys		Pro Leu Glu Leu Ala		Ile Phe Asp Leu Arg
	Pro His Ala Lys Lys		Leu Glu Leu Ala Gly		Phe Asp Leu Arg Gly
	His Ala Lys Lys Lys		Glu Leu Ala Gly Leu		Asp Leu Arg Gly Lys
	Ala Lys Lys Lys Ser		Leu Ala Gly Leu Gly		Leu Arg Gly Lys Phe
	Lys Lys Lys Ser Lys		Ala Gly Leu Gly Phe		Arg Gly Lys Phe Lys
	Lys Lys Ser Lys Ile		Gly Leu Gly Phe Ala		Gly Lys Phe Lys Arg
	Lys Ser Lys Ile Ser		Leu Gly Phe Ala Glu		Lys Phe Lys Arg Pro
	Ser Lys Ile Ser Ala		Gly Phe Ala Glu Leu		Phe Lys Arg Pro Thr
	Lys Ile Ser Ala Ser		Phe Ala Glu Leu Gln		Lys Arg Pro Thr Leu
	Ile Ser Ala Ser Arg		Ala Glu Leu Gln Asp		Arg Pro Thr Leu Arg
	Ser Ala Ser Arg Lys		Glu Leu Gln Asp Leu		
	Ala Ser Arg Lys Leu		Leu Gln Asp Leu Cys		
	Ser Arg Lys Leu Gln		Gln Asp Leu Cys Arg		
	Arg Lys Leu Gln Leu		Asp Leu Cys Arg Gln		
	Lys Leu Gln Leu Lys		Leu Cys Arg Gln Leu		
	Leu Gln Leu Lys Thr		Cys Arg Gln Leu His		
	Gln Leu Lys Thr Leu		Arg Gln Leu His Ala		
	Leu Lys Thr Leu Leu		Gln Leu His Ala Arg		
	Lys Thr Leu Leu Leu		Leu His Ala Arg Val		
	Thr Leu Leu Leu Gln		His Ala Arg Val Asp		

When cardiac troponin T (SEQ ID NO:2) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of cardiac troponin T (SEQ ID NO: 2). Table 3 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from cardiac troponin T (SEQ ID NO: 2). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 3, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID

NO:2, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 3:

	Ser Asp Ile Glu Glu		Arg Ala Glu Glu Asp
	Asp Ile Glu Glu Val		Ala Glu Glu Asp Glu
	Ile Glu Glu Val Val		Glu Glu Asp Glu Glu
	Glu Glu Val Val Glu		Glu Asp Glu Glu Glu
	Glu Val Val Glu Glu		Asp Glu Glu Glu Glu
	Val Val Glu Glu Tyr		Glu Glu Glu Glu Glu
	Val Glu Glu Tyr Glu		Glu Glu Glu Glu Ala
	Glu Glu Tyr Glu Glu		Glu Glu Glu Ala Lys
	Glu Tyr Glu Glu Glu		Glu Glu Ala Lys Glu
	Tyr Glu Glu Glu Glu		Glu Ala Lys Glu Ala
	Glu Glu Glu Glu Gln		Ala Lys Glu Ala Glu
	Glu Glu Glu Gln Glu		Lys Glu Ala Glu Asp
	Glu Glu Gln Glu Glu		Glu Ala Glu Asp Gly
	Glu Gln Glu Glu Ala		Ala Glu Asp Gly Pro
	Gln Glu Glu Ala Ala		Glu Asp Gly Pro Met
	Glu Glu Ala Ala Val		Asp Gly Pro Met Glu
	Glu Ala Ala Val Glu		Gly Pro Met Glu Glu
	Ala Ala Val Glu Glu		Pro Met Glu Glu Ser
	Ala Val Glu Glu Glu		Met Glu Glu Ser Lys
	Val Glu Glu Glu Glu		Glu Glu Ser Lys Pro
	Glu Glu Glu Glu Asp		Glu Ser Lys Pro Lys
	Glu Glu Glu Asp Trp		Ser Lys Pro Lys Pro
	Glu Glu Asp Trp Arg		Lys Pro Lys Pro Arg
	Glu Asp Trp Arg Glu		Pro Lys Pro Arg Ser
	Asp Trp Arg Glu Asp		Lys Pro Arg Ser Phe
	Trp Arg Glu Asp Glu		Pro Arg Ser Phe Met
	Arg Glu Asp Glu Asp		
	Glu Asp Glu Asp Glu		
	Asp Glu Asp Glu Gln		

	Glu Asp Glu Gln Glu		
	Asp Glu Gln Glu Glu		
	Glu Gln Glu Glu Ala		
	Gln Glu Glu Ala Ala		
	Glu Glu Ala Ala Glu		
	Glu Ala Ala Glu Glu		
	Ala Ala Glu Glu Asp		
	Ala Glu Glu Asp Ala		
	Glu Glu Asp Ala Glu		
	Glu Asp Ala Glu Ala		
	Asp Ala Glu Ala Glu		
	Ala Glu Ala Glu Ala		
	Glu Ala Glu Ala Glu		
	Ala Glu Ala Glu Thr		
	Glu Ala Glu Thr Glu		
	Ala Glu Thr Glu Glu		
	Glu Thr Glu Glu Thr		
	Thr Glu Glu Thr Arg		
	Glu Glu Thr Arg Ala		
	Glu Thr Arg Ala Glu		
	Thr Arg Ala Glu Glu		

When thyroid stimulating hormone (TSH) (SEQ ID NO:3) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of (TSH) (SEQ ID NO:3). Table 3 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from TSH (SEQ ID NO: 3). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 4, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:3, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 4:

	Thr Ala Leu Phe Leu		Met Thr Arg Asp Ile		Ser Cys Lys Cys Gly
	Ala Leu Phe Leu Met		Thr Arg Asp Ile Asn		Cys Lys Cys Gly Lys
	Leu Phe Leu Met Ser		Arg Asp Ile Asn Gly		Lys Cys Gly Lys Cys
	Phe Leu Met Ser Met		Asp Ile Asn Gly Lys		Cys Gly Lys Cys Asn
	Leu Met Ser Met Leu		Ile Asn Gly Lys Leu		Gly Lys Cys Asn Thr
	Met Ser Met Leu Phe		Asn Gly Lys Leu Phe		Lys Cys Asn Thr Asp
	Ser Met Leu Phe Gly		Gly Lys Leu Phe Leu		Cys Asn Thr Asp Tyr
	Met Leu Phe Gly Leu		Lys Leu Phe Leu Pro		Asn Thr Asp Tyr Ser
	Leu Phe Gly Leu Ala		Leu Phe Leu Pro Lys		Thr Asp Tyr Ser Asp
	Phe Gly Leu Ala Cys		Phe Leu Pro Lys Tyr		Asp Tyr Ser Asp Cys
	Gly Leu Ala Cys Gly		Leu Pro Lys Tyr Ala		Tyr Ser Asp Cys Ile
	Leu Ala Cys Gly Gln		Pro Lys Tyr Ala Leu		Ser Asp Cys Ile His
	Ala Cys Gly Gln Ala		Lys Tyr Ala Leu Ser		Asp Cys Ile His Glu
	Cys Gly Gln Ala Met		Tyr Ala Leu Ser Gln		Cys Ile His Glu Ala
	Gly Gln Ala Met Ser		Ala Leu Ser Gln Asp		Ile His Glu Ala Ile
	Gln Ala Met Ser Phe		Leu Ser Gln Asp Val		His Glu Ala Ile Lys
	Ala Met Ser Phe Cys		Ser Gln Asp Val Cys		Glu Ala Ile Lys Thr
	Met Ser Phe Cys Ile		Gln Asp Val Cys Thr		Ala Ile Lys Thr Asn
	Ser Phe Cys Ile Pro		Asp Val Cys Thr Tyr		Ile Lys Thr Asn Tyr
	Phe Cys Ile Pro Thr		Val Cys Thr Tyr Arg		Lys Thr Asn Tyr Cys
	Cys Ile Pro Thr Glu		Cys Thr Tyr Arg Asp		Thr Asn Tyr Cys Thr
	Ile Pro Thr Glu Tyr		Thr Tyr Arg Asp Phe		Asn Tyr Cys Thr Lys
	Pro Thr Glu Tyr Thr		Tyr Arg Asp Phe Ile		Tyr Cys Thr Lys Pro
	Thr Glu Tyr Thr Met		Arg Asp Phe Ile Tyr		Cys Thr Lys Pro Gln
	Glu Tyr Thr Met His		Asp Phe Ile Tyr Arg		Thr Lys Pro Gln Lys
	Tyr Thr Met His Ile		Phe Ile Tyr Arg Thr		Lys Pro Gln Lys Ser
	Thr Met His Ile Glu		Ile Tyr Arg Thr Val		Pro Gln Lys Ser Tyr
	Met His Ile Glu Arg		Tyr Arg Thr Val Glu		Gln Lys Ser Tyr Leu
	His Ile Glu Arg Arg		Arg Thr Val Glu Ile		Lys Ser Tyr Leu Val
	Ile Glu Arg Arg Glu		Thr Val Glu Ile Pro		Ser Tyr Leu Val Gly
	Glu Arg Arg Glu Cys		Val Glu Ile Pro Gly		Tyr Leu Val Gly Phe
	Arg Arg Glu Cys Ala		Glu Ile Pro Gly Cys		Leu Val Gly Phe Ser

	Arg Glu Cys Ala Tyr		Ile Pro Gly Cys Pro		Val Gly Phe Ser Val
	Glu Cys Ala Tyr Cys		Pro Gly Cys Pro Leu		
	Cys Ala Tyr Cys Leu		Gly Cys Pro Leu His		
	Ala Tyr Cys Leu Thr		Cys Pro Leu His Val		
	Tyr Cys Leu Thr Ile		Pro Leu His Val Ala		
	Cys Leu Thr Ile Asn		Leu His Val Ala Pro		
	Leu Thr Ile Asn Thr		His Val Ala Pro Tyr		
	Thr Ile Asn Thr Thr		Val Ala Pro Tyr Phe		
	Ile Asn Thr Thr Ile		Ala Pro Tyr Phe Ser		
	Asn Thr Thr Ile Cys		Pro Tyr Phe Ser Tyr		
	Thr Thr Ile Cys Ala		Tyr Phe Ser Tyr Pro		
	Thr Ile Cys Ala Gly		Phe Ser Tyr Pro Val		
	Ile Cys Ala Gly Tyr		Ser Tyr Pro Val Ala		
	Cys Ala Gly Tyr Cys		Tyr Pro Val Ala Leu		
	Ala Gly Tyr Cys Met		Pro Val Ala Leu Ser		
	Gly Tyr Cys Met Thr		Val Ala Leu Ser Cys		
	Tyr Cys Met Thr Arg		Ala Leu Ser Cys Lys		
	Cys Met Thr Arg Asp		Leu Ser Cys Lys Cys		

When the beta subunit of human chorionic gonadotropin (beta-HCG) (SEQ ID NO:4) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of beta-HCG (SEQ ID NO:4). Table 5 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from beta-HCG (SEQ ID NO: 4). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 5, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:4, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 5:

	Glu Met Phe Gln Gly		Thr Ile Cys Ala Gly		Tyr Ala Val Ala Leu
	Met Phe Gln Gly Leu		Ile Cys Ala Gly Tyr		Ala Val Ala Leu Ser
	Phe Gln Gly Leu Leu		Cys Ala Gly Tyr Cys		Val Ala Leu Ser Cys

	Gln Gly Leu Leu Leu		Ala Gly Tyr Cys Pro		Ala Leu Ser Cys Gln
	Gly Leu Leu Leu Leu		Gly Tyr Cys Pro Thr		Leu Ser Cys Gln Cys
	Leu Leu Leu Leu Leu		Tyr Cys Pro Thr Met		Ser Cys Gln Cys Ala
	Leu Leu Leu Leu Leu		Cys Pro Thr Met Thr		Cys Gln Cys Ala Leu
	Leu Leu Leu Leu Leu		Pro Thr Met Thr Arg		Gln Cys Ala Leu Cys
	Leu Leu Leu Leu Ser		Thr Met Thr Arg Val		Cys Ala Leu Cys Arg
	Leu Leu Leu Ser Met		Met Thr Arg Val Leu		Ala Leu Cys Arg Arg
	Leu Leu Ser Met Gly		Thr Arg Val Leu Gln		Leu Cys Arg Arg Ser
	Leu Ser Met Gly Gly		Arg Val Leu Gln Gly		Cys Arg Arg Ser Thr
	Ser Met Gly Gly Thr		Val Leu Gln Gly Val		Arg Arg Ser Thr Thr
	Met Gly Gly Thr Trp		Leu Gln Gly Val Leu		Arg Ser Thr Thr Asp
	Gly Gly Thr Trp Ala		Gln Gly Val Leu Pro		Ser Thr Thr Asp Cys
	Gly Thr Trp Ala Ser		Gly Val Leu Pro Ala		Thr Thr Asp Cys Gly
	Thr Trp Ala Ser Lys		Val Leu Pro Ala Leu		Thr Asp Cys Gly Gly
	Trp Ala Ser Lys Glu		Leu Pro Ala Leu Pro		Asp Cys Gly Gly Pro
	Ala Ser Lys Glu Pro		Pro Ala Leu Pro Gln		Cys Gly Gly Pro Lys
	Ser Lys Glu Pro Leu		Ala Leu Pro Gln Val		Gly Gly Pro Lys Asp
	Lys Glu Pro Leu Arg		Leu Pro Gln Val Val		Gly Pro Lys Asp His
	Glu Pro Leu Arg Pro		Pro Gln Val Val Cys		Pro Lys Asp His Pro
	Pro Leu Arg Pro Arg		Gln Val Val Cys Asn		Lys Asp His Pro Leu
	Leu Arg Pro Arg Cys		Val Val Cys Asn Tyr		Asp His Pro Leu Thr
	Arg Pro Arg Cys Arg		Val Cys Asn Tyr Arg		His Pro Leu Thr Cys
	Pro Arg Cys Arg Pro		Cys Asn Tyr Arg Asp		Pro Leu Thr Cys Asp
	Arg Cys Arg Pro Ile		Asn Tyr Arg Asp Val		Leu Thr Cys Asp Asp
	Cys Arg Pro Ile Asn		Tyr Arg Asp Val Arg		Thr Cys Asp Asp Pro
	Arg Pro Ile Asn Ala		Arg Asp Val Arg Phe		Cys Asp Asp Pro Arg
	Pro Ile Asn Ala Thr		Asp Val Arg Phe Glu		Asp Asp Pro Arg Phe
	Ile Asn Ala Thr Leu		Val Arg Phe Glu Ser		Asp Pro Arg Phe Gln
	Asn Ala Thr Leu Ala		Arg Phe Glu Ser Ile		Pro Arg Phe Gln Asp
	Ala Thr Leu Ala Val		Phe Glu Ser Ile Arg		Arg Phe Gln Asp Ser
	Thr Leu Ala Val Glu		Glu Ser Ile Arg Leu		Phe Gln Asp Ser Ser
	Leu Ala Val Glu Lys		Ser Ile Arg Leu Pro		Gln Asp Ser Ser Ser

	Ala Val Glu Lys Glu		Ile Arg Leu Pro Gly		Asp Ser Ser Ser Ser
	Val Glu Lys Glu Gly		Arg Leu Pro Gly Cys		Ser Ser Ser Ser Lys
	Glu Lys Glu Gly Cys		Leu Pro Gly Cys Pro		Ser Ser Ser Lys Ala
	Lys Glu Gly Cys Pro		Pro Gly Cys Pro Arg		Ser Ser Lys Ala Pro
	Glu Gly Cys Pro Val		Gly Cys Pro Arg Gly		Ser Lys Ala Pro Pro
	Gly Cys Pro Val Cys		Cys Pro Arg Gly Val		
	Cys Pro Val Cys Ile		Pro Arg Gly Val Asn		
	Pro Val Cys Ile Thr		Arg Gly Val Asn Pro		
	Val Cys Ile Thr Val		Gly Val Asn Pro Val		
	Cys Ile Thr Val Asn		Val Asn Pro Val Val		
	Ile Thr Val Asn Thr		Asn Pro Val Val Ser		
	Thr Val Asn Thr Thr		Pro Val Val Ser Tyr		
	Val Asn Thr Thr Ile		Val Val Ser Tyr Ala		
	Asn Thr Thr Ile Cys		Val Ser Tyr Ala Val		
	Thr Thr Ile Cys Ala		Ser Tyr Ala Val Ala		

When myeloperoxidase (MPO) (SEQ ID NO:5) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of MPO (SEQ ID NO:5). Table 6 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from MPO (SEQ ID NO: 5). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 6, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:5, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 6:

	Gly Val Pro Phe Phe		Ala Val Leu Gly Glu		Phe Lys Gln Pro Val
	Val Pro Phe Phe Ser		Val Leu Gly Glu Val		Lys Gln Pro Val Ala
	Pro Phe Phe Ser Ser		Leu Gly Glu Val Asp		Gln Pro Val Ala Ala
	Phe Phe Ser Ser Leu		Gly Glu Val Asp Thr		Pro Val Ala Ala Thr
	Phe Ser Ser Leu Arg		Glu Val Asp Thr Ser		Val Ala Ala Thr Arg
	Ser Ser Leu Arg Cys		Val Asp Thr Ser Leu		Ala Ala Thr Arg Thr
	Ser Leu Arg Cys Met		Asp Thr Ser Leu Val		Ala Thr Arg Thr Ala

	Leu Arg Cys Met Val		Thr Ser Leu Val Leu		Thr Arg Thr Ala Val
	Arg Cys Met Val Asp		Ser Leu Val Leu Ser		Arg Thr Ala Val Arg
	Cys Met Val Asp Leu		Leu Val Leu Ser Ser		Thr Ala Val Arg Ala
	Met Val Asp Leu Gly		Val Leu Ser Ser Met		Ala Val Arg Ala Ala
	Val Asp Leu Gly Pro		Leu Ser Ser Met Glu		Val Arg Ala Ala Asp
	Asp Leu Gly Pro Cys		Ser Ser Met Glu Glu		Arg Ala Ala Asp Tyr
	Leu Gly Pro Cys Trp		Ser Met Glu Glu Ala		Ala Ala Asp Tyr Leu
	Gly Pro Cys Trp Ala		Met Glu Glu Ala Lys		Ala Asp Tyr Leu His
	Pro Cys Trp Ala Gly		Glu Glu Ala Lys Gln		Asp Tyr Leu His Val
	Cys Trp Ala Gly Gly		Glu Ala Lys Gln Leu		Tyr Leu His Val Ala
	Trp Ala Gly Gly Leu		Ala Lys Gln Leu Val		Leu His Val Ala Leu
	Ala Gly Gly Leu Thr		Lys Gln Leu Val Asp		His Val Ala Leu Asp
	Gly Gly Leu Thr Ala		Gln Leu Val Asp Lys		Val Ala Leu Asp Leu
	Gly Leu Thr Ala Glu		Leu Val Asp Lys Ala		Ala Leu Asp Leu Leu
	Leu Thr Ala Glu Met		Val Asp Lys Ala Tyr		Leu Asp Leu Leu Glu
	Thr Ala Glu Met Lys		Asp Lys Ala Tyr Lys		Asp Leu Leu Glu Arg
	Ala Glu Met Lys Leu		Lys Ala Tyr Lys Glu		Leu Leu Glu Arg Lys
	Glu Met Lys Leu Leu		Ala Tyr Lys Glu Arg		Leu Glu Arg Lys Leu
	Met Lys Leu Leu Leu		Tyr Lys Glu Arg Arg		Glu Arg Lys Leu Arg
	Lys Leu Leu Leu Ala		Lys Glu Arg Arg Glu		Arg Lys Leu Arg Ser
	Leu Leu Leu Ala Leu		Glu Arg Arg Glu Ser		Lys Leu Arg Ser Leu
	Leu Leu Ala Leu Ala		Arg Arg Glu Ser Ile		Leu Arg Ser Leu Trp
	Leu Ala Leu Ala Gly		Arg Glu Ser Ile Lys		Arg Ser Leu Trp Arg
	Ala Leu Ala Gly Leu		Glu Ser Ile Lys Gln		Ser Leu Trp Arg Arg
	Leu Ala Gly Leu Leu		Ser Ile Lys Gln Arg		Leu Trp Arg Arg Pro
	Ala Gly Leu Leu Ala		Ile Lys Gln Arg Leu		Trp Arg Arg Pro Phe
	Gly Leu Leu Ala Ile		Lys Gln Arg Leu Arg		Arg Arg Pro Phe Asn
	Leu Leu Ala Ile Leu		Gln Arg Leu Arg Ser		Arg Pro Phe Asn Val
	Leu Ala Ile Leu Ala		Arg Leu Arg Ser Gly		Pro Phe Asn Val Thr
	Ala Ile Leu Ala Thr		Leu Arg Ser Gly Ser		Phe Asn Val Thr Asp
	Ile Leu Ala Thr Pro		Arg Ser Gly Ser Ala		Asn Val Thr Asp Val
	Leu Ala Thr Pro Gln		Ser Gly Ser Ala Ser		Val Thr Asp Val Leu

	Ala Thr Pro Gln Pro		Gly Ser Ala Ser Pro		Thr Asp Val Leu Thr
	Thr Pro Gln Pro Ser		Ser Ala Ser Pro Met		Asp Val Leu Thr Pro
	Pro Gln Pro Ser Glu		Ala Ser Pro Met Glu		Val Leu Thr Pro Ala
	Gln Pro Ser Glu Gly		Ser Pro Met Glu Leu		Leu Thr Pro Ala Gln
	Pro Ser Glu Gly Ala		Pro Met Glu Leu Leu		Thr Pro Ala Gln Leu
	Ser Glu Gly Ala Ala		Met Glu Leu Leu Ser		Pro Ala Gln Leu Asn
	Glu Gly Ala Ala Pro		Glu Leu Leu Ser Tyr		Ala Gln Leu Asn Val
	Gly Ala Ala Pro Ala		Leu Leu Ser Tyr Phe		Gln Leu Asn Val Leu
	Ala Ala Pro Ala Val		Leu Ser Tyr Phe Lys		Leu Asn Val Leu Ser
	Ala Pro Ala Val Leu		Ser Tyr Phe Lys Gln		Asn Val Leu Ser Lys
	Pro Ala Val Leu Gly		Tyr Phe Lys Gln Pro		Val Leu Ser Lys Ser
	Leu Ser Lys Ser Ser		Glu Asp Gly Phe Ser		
	Ser Lys Ser Ser Gly		Asp Gly Phe Ser Leu		
	Lys Ser Ser Gly Cys		Gly Phe Ser Leu Pro		
	Ser Ser Gly Cys Ala		Phe Ser Leu Pro Tyr		
	Ser Gly Cys Ala Tyr		Ser Leu Pro Tyr Gly		
	Gly Cys Ala Tyr Gln		Leu Pro Tyr Gly Trp		
	Cys Ala Tyr Gln Asp		Pro Tyr Gly Trp Thr		
	Ala Tyr Gln Asp Val		Tyr Gly Trp Thr Pro		
	Tyr Gln Asp Val Gly		Gly Trp Thr Pro Gly		
	Gln Asp Val Gly Val		Trp Thr Pro Gly Val		
	Asp Val Gly Val Thr		Thr Pro Gly Val Lys		
	Val Gly Val Thr Cys		Pro Gly Val Lys Arg		
	Gly Val Thr Cys Pro		Gly Val Lys Arg Asn		
	Val Thr Cys Pro Glu		Val Lys Arg Asn Gly		
	Thr Cys Pro Glu Gln		Lys Arg Asn Gly Phe		
	Cys Pro Glu Gln Asp		Arg Asn Gly Phe Pro		
	Pro Glu Gln Asp Lys		Asn Gly Phe Pro Val		
	Glu Gln Asp Lys Tyr		Gly Phe Pro Val Ala		
	Gln Asp Lys Tyr Arg				
	Asp Lys Tyr Arg Thr				
	Lys Tyr Arg Thr Ile				

	Tyr Arg Thr Ile Thr				
	Arg Thr Ile Thr Gly				
	Thr Ile Thr Gly Met				
	Ile Thr Gly Met Cys				
	Thr Gly Met Cys Asn				
	Gly Met Cys Asn Asn				
	Met Cys Asn Asn Arg				
	Cys Asn Asn Arg Arg				
	Asn Asn Arg Arg Ser				
	Asn Arg Arg Ser Pro				
	Arg Arg Ser Pro Thr				
	Arg Ser Pro Thr Leu				
	Ser Pro Thr Leu Gly				
	Pro Thr Leu Gly Ala				
	Thr Leu Gly Ala Ser				
	Leu Gly Ala Ser Asn				
	Gly Ala Ser Asn Arg				
	Ala Ser Asn Arg Ala				
	Ser Asn Arg Ala Phe				
	Asn Arg Ala Phe Val				
	Arg Ala Phe Val Arg				
	Ala Phe Val Arg Trp				
	Phe Val Arg Trp Leu				
	Val Arg Trp Leu Pro				
	Arg Trp Leu Pro Ala				
	Trp Leu Pro Ala Glu				
	Leu Pro Ala Glu Tyr				
	Pro Ala Glu Tyr Glu				
	Ala Glu Tyr Glu Asp				
	Glu Tyr Glu Asp Gly				
	Tyr Glu Asp Gly Phe				

When prostate specific antigen (PSA) (SEQ ID NO:6) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of PSA (SEQ ID NO: 6). Table 6 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from PSA (SEQ ID NO: 6). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 7, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:6, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 7:

	Trp Val Pro Val Val		Gly Val Leu Val His		Asp Met Ser Leu Leu
	Val Pro Val Val Phe		Val Leu Val His Pro		Met Ser Leu Leu Lys
	Pro Val Val Phe Leu		Leu Val His Pro Gln		Ser Leu Leu Lys Asn
	Val Val Phe Leu Thr		Val His Pro Gln Trp		Leu Leu Lys Asn Arg
	Val Phe Leu Thr Leu		His Pro Gln Trp Val		Leu Lys Asn Arg Phe
	Phe Leu Thr Leu Ser		Pro Gln Trp Val Leu		Lys Asn Arg Phe Leu
	Leu Thr Leu Ser Val		Gln Trp Val Leu Thr		Asn Arg Phe Leu Arg
	Thr Leu Ser Val Thr		Trp Val Leu Thr Ala		Arg Phe Leu Arg Pro
	Leu Ser Val Thr Trp		Val Leu Thr Ala Ala		Phe Leu Arg Pro Gly
	Ser Val Thr Trp Ile		Leu Thr Ala Ala His		Leu Arg Pro Gly Asp
	Val Thr Trp Ile Gly		Thr Ala Ala His Cys		Arg Pro Gly Asp Asp
	Thr Trp Ile Gly Ala		Ala Ala His Cys Ile		Pro Gly Asp Asp Ser
	Trp Ile Gly Ala Ala		Ala His Cys Ile Arg		Gly Asp Asp Ser Ser
	Ile Gly Ala Ala Pro		His Cys Ile Arg Asn		Asp Asp Ser Ser His
	Gly Ala Ala Pro Leu		Cys Ile Arg Asn Lys		Asp Ser Ser His Asp
	Ala Ala Pro Leu Ile		Ile Arg Asn Lys Ser		Ser Ser His Asp Leu
	Ala Pro Leu Ile Leu		Arg Asn Lys Ser Val		Ser His Asp Leu Met
	Pro Leu Ile Leu Ser		Asn Lys Ser Val Ile		His Asp Leu Met Leu
	Leu Ile Leu Ser Arg		Lys Ser Val Ile Leu		Asp Leu Met Leu Leu
	Ile Leu Ser Arg Ile		Ser Val Ile Leu Leu		Leu Met Leu Leu Arg
	Leu Ser Arg Ile Val		Val Ile Leu Leu Gly		Met Leu Leu Arg Leu
	Ser Arg Ile Val Gly		Ile Leu Leu Gly Arg		Leu Leu Arg Leu Ser

	Arg Ile Val Gly Gly		Leu Leu Gly Arg His		Leu Arg Leu Ser Glu
	Ile Val Gly Gly Trp		Leu Gly Arg His Ser		Arg Leu Ser Glu Pro
	Val Gly Gly Trp Glu		Gly Arg His Ser Leu		Leu Ser Glu Pro Ala
	Gly Gly Trp Glu Cys		Arg His Ser Leu Phe		Ser Glu Pro Ala Glu
	Gly Trp Glu Cys Glu		His Ser Leu Phe His		Glu Pro Ala Glu Leu
	Trp Glu Cys Glu Lys		Ser Leu Phe His Pro		Pro Ala Glu Leu Thr
	Glu Cys Glu Lys His		Leu Phe His Pro Glu		Ala Glu Leu Thr Asp
	Cys Glu Lys His Ser		Phe His Pro Glu Asp		Glu Leu Thr Asp Ala
	Glu Lys His Ser Gln		His Pro Glu Asp Thr		Leu Thr Asp Ala Val
	Lys His Ser Gln Pro		Pro Glu Asp Thr Gly		Thr Asp Ala Val Lys
	His Ser Gln Pro Trp		Glu Asp Thr Gly Gln		Asp Ala Val Lys Val
	Ser Gln Pro Trp Gln		Asp Thr Gly Gln Val		Ala Val Lys Val Met
	Gln Pro Trp Gln Val		Thr Gly Gln Val Phe		Val Lys Val Met Asp
	Pro Trp Gln Val Leu		Gly Gln Val Phe Gln		Lys Val Met Asp Leu
	Trp Gln Val Leu Val		Gln Val Phe Gln Val		Val Met Asp Leu Pro
	Gln Val Leu Val Ala		Val Phe Gln Val Ser		Met Asp Leu Pro Thr
	Val Leu Val Ala Ser		Phe Gln Val Ser His		Asp Leu Pro Thr Gln
	Leu Val Ala Ser Arg		Gln Val Ser His Ser		Leu Pro Thr Gln Glu
	Val Ala Ser Arg Gly		Val Ser His Ser Phe		Pro Thr Gln Glu Pro
	Ala Ser Arg Gly Arg		Ser His Ser Phe Pro		Thr Gln Glu Pro Ala
	Ser Arg Gly Arg Ala		His Ser Phe Pro His		Gln Glu Pro Ala Leu
	Arg Gly Arg Ala Val		Ser Phe Pro His Pro		Glu Pro Ala Leu Gly
	Gly Arg Ala Val Cys		Phe Pro His Pro Leu		Pro Ala Leu Gly Thr
	Arg Ala Val Cys Gly		Pro His Pro Leu Tyr		Ala Leu Gly Thr Thr
	Ala Val Cys Gly Gly		His Pro Leu Tyr Asp		Leu Gly Thr Thr Cys
	Val Cys Gly Gly Val		Pro Leu Tyr Asp Met		Gly Thr Thr Cys Tyr
	Cys Gly Gly Val Leu		Leu Tyr Asp Met Ser		Thr Thr Cys Tyr Ala
	Gly Gly Val Leu Val		Tyr Asp Met Ser Leu		Thr Cys Tyr Ala Ser
	Cys Tyr Ala Ser Gly		Gly Gly Lys Ser Thr		Thr Ile Val Ala Asn
	Tyr Ala Ser Gly Trp		Gly Lys Ser Thr Cys		Ile Val Ala Asn Pro
	Ala Ser Gly Trp Gly		Lys Ser Thr Cys Ser		
	Ser Gly Trp Gly Ser		Ser Thr Cys Ser Gly		

	Gly Trp Gly Ser Ile		Thr Cys Ser Gly Asp		
	Trp Gly Ser Ile Glu		Cys Ser Gly Asp Ser		
	Gly Ser Ile Glu Pro		Ser Gly Asp Ser Gly		
	Ser Ile Glu Pro Glu		Gly Asp Ser Gly Gly		
	Ile Glu Pro Glu Glu		Asp Ser Gly Gly Pro		
	Glu Pro Glu Glu Phe		Ser Gly Gly Pro Leu		
	Pro Glu Glu Phe Leu		Gly Gly Pro Leu Val		
	Glu Glu Phe Leu Thr		Gly Pro Leu Val Cys		
	Glu Phe Leu Thr Pro		Pro Leu Val Cys Asn		
	Phe Leu Thr Pro Lys		Leu Val Cys Asn Gly		
	Leu Thr Pro Lys Lys		Val Cys Asn Gly Val		
	Thr Pro Lys Lys Leu		Cys Asn Gly Val Leu		
	Pro Lys Lys Leu Gln		Asn Gly Val Leu Gln		
	Lys Lys Leu Gln Cys		Gly Val Leu Gln Gly		
	Lys Leu Gln Cys Val		Val Leu Gln Gly Ile		
	Leu Gln Cys Val Asp		Leu Gln Gly Ile Thr		
	Gln Cys Val Asp Leu		Gln Gly Ile Thr Ser		
	Cys Val Asp Leu His		Gly Ile Thr Ser Trp		
	Val Asp Leu His Val		Ile Thr Ser Trp Gly		
	Asp Leu His Val Ile		Thr Ser Trp Gly Ser		
	Leu His Val Ile Ser		Ser Trp Gly Ser Glu		
	His Val Ile Ser Asn		Trp Gly Ser Glu Pro		
	Val Ile Ser Asn Asp		Gly Ser Glu Pro Cys		
	Ile Ser Asn Asp Val		Ser Glu Pro Cys Ala		
	Ser Asn Asp Val Cys		Glu Pro Cys Ala Leu		
	Asn Asp Val Cys Ala		Pro Cys Ala Leu Pro		
	Asp Val Cys Ala Gln		Cys Ala Leu Pro Glu		
	Val Cys Ala Gln Val		Ala Leu Pro Glu Arg		
	Cys Ala Gln Val His		Leu Pro Glu Arg Pro		
	Ala Gln Val His Pro		Pro Glu Arg Pro Ser		
	Gln Val His Pro Gln		Glu Arg Pro Ser Leu		
	Val His Pro Gln Lys		Arg Pro Ser Leu Tyr		

	His Pro Gln Lys Val		Pro Ser Leu Tyr Thr		
	Pro Gln Lys Val Thr		Ser Leu Tyr Thr Lys		
	Gln Lys Val Thr Lys		Leu Tyr Thr Lys Val		
	Lys Val Thr Lys Phe		Tyr Thr Lys Val Val		
	Val Thr Lys Phe Met		Thr Lys Val Val His		
	Thr Lys Phe Met Leu		Lys Val Val His Tyr		
	Lys Phe Met Leu Cys		Val Val His Tyr Arg		
	Phe Met Leu Cys Ala		Val His Tyr Arg Lys		
	Met Leu Cys Ala Gly		His Tyr Arg Lys Trp		
	Leu Cys Ala Gly Arg		Tyr Arg Lys Trp Ile		
	Cys Ala Gly Arg Trp		Arg Lys Trp Ile Lys		
	Ala Gly Arg Trp Thr		Lys Trp Ile Lys Asp		
	Gly Arg Trp Thr Gly		Trp Ile Lys Asp Thr		
	Arg Trp Thr Gly Gly		Ile Lys Asp Thr Ile		
	Trp Thr Gly Gly Lys		Lys Asp Thr Ile Val		
	Thr Gly Gly Lys Ser		Asp Thr Ile Val Ala		

When human B-type natriuretic peptide (hBNP) (SEQ ID NO:7) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of hBNP (SEQ ID NO:7). Table 8 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from hBNP (SEQ ID NO: 7). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 8, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:7, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 8:

	Asp Pro Gln Thr Ala		Gly Lys Leu Ser Glu		Arg Ser Pro Lys Met
	Pro Gln Thr Ala Pro		Lys Leu Ser Glu Leu		Ser Pro Lys Met Val
	Gln Thr Ala Pro Ser		Leu Ser Glu Leu Gln		Pro Lys Met Val Gln
	Thr Ala Pro Ser Arg		Ser Glu Leu Gln Val		Lys Met Val Gln Gly
	Ala Pro Ser Arg Ala		Glu Leu Gln Val Glu		Met Val Gln Gly Ser

	Pro Ser Arg Ala Leu		Leu Gln Val Glu Gln		Val Gln Gly Ser Gly
	Ser Arg Ala Leu Leu		Gln Val Glu Gln Thr		Gln Gly Ser Gly Cys
	Arg Ala Leu Leu Leu		Val Glu Gln Thr Ser		Gly Ser Gly Cys Phe
	Ala Leu Leu Leu Leu		Glu Gln Thr Ser Leu		Ser Gly Cys Phe Gly
	Leu Leu Leu Leu Leu		Gln Thr Ser Leu Glu		Gly Cys Phe Gly Arg
	Leu Leu Leu Leu Phe		Thr Ser Leu Glu Pro		Cys Phe Gly Arg Lys
	Leu Leu Leu Phe Leu		Ser Leu Glu Pro Leu		Phe Gly Arg Lys Met
	Leu Leu Phe Leu His		Leu Glu Pro Leu Gln		Gly Arg Lys Met Asp
	Leu Phe Leu His Leu		Glu Pro Leu Gln Glu		Arg Lys Met Asp Arg
	Phe Leu His Leu Ala		Pro Leu Gln Glu Ser		Lys Met Asp Arg Ile
	Leu His Leu Ala Phe		Leu Gln Glu Ser Pro		Met Asp Arg Ile Ser
	His Leu Ala Phe Leu		Gln Glu Ser Pro Arg		Asp Arg Ile Ser Ser
	Leu Ala Phe Leu Gly		Glu Ser Pro Arg Pro		Arg Ile Ser Ser Ser
	Ala Phe Leu Gly Gly		Ser Pro Arg Pro Thr		Ile Ser Ser Ser Ser
	Phe Leu Gly Gly Arg		Pro Arg Pro Thr Gly		Ser Ser Ser Ser Gly
	Leu Gly Gly Arg Ser		Arg Pro Thr Gly Val		Ser Ser Ser Gly Leu
	Gly Gly Arg Ser His		Pro Thr Gly Val Trp		Ser Ser Gly Leu Gly
	Gly Arg Ser His Pro		Thr Gly Val Trp Lys		Ser Gly Leu Gly Cys
	Arg Ser His Pro Leu		Gly Val Trp Lys Ser		Gly Leu Gly Cys Lys
	Ser His Pro Leu Gly		Val Trp Lys Ser Arg		Leu Gly Cys Lys Val
	His Pro Leu Gly Ser		Trp Lys Ser Arg Glu		Gly Cys Lys Val Leu
	Pro Leu Gly Ser Pro		Lys Ser Arg Glu Val		Cys Lys Val Leu Arg
	Leu Gly Ser Pro Gly		Ser Arg Glu Val Ala		Lys Val Leu Arg Arg
	Gly Ser Pro Gly Ser		Arg Glu Val Ala Thr		Val Leu Arg Arg His
	Ser Pro Gly Ser Ala		Glu Val Ala Thr Glu		
	Pro Gly Ser Ala Ser		Val Ala Thr Glu Gly		
	Gly Ser Ala Ser Asp		Ala Thr Glu Gly Ile		
	Ser Ala Ser Asp Leu		Thr Glu Gly Ile Arg		
	Ala Ser Asp Leu Glu		Glu Gly Ile Arg Gly		
	Ser Asp Leu Glu Thr		Gly Ile Arg Gly His		
	Asp Leu Glu Thr Ser		Ile Arg Gly His Arg		
	Leu Glu Thr Ser Gly		Arg Gly His Arg Lys		

	Glu Thr Ser Gly Leu		Gly His Arg Lys Met		
	Thr Ser Gly Leu Gln		His Arg Lys Met Val		
	Ser Gly Leu Gln Glu		Arg Lys Met Val Leu		
	Gly Leu Gln Glu Gln		Lys Met Val Leu Tyr		
	Leu Gln Glu Gln Arg		Met Val Leu Tyr Thr		
	Gln Glu Gln Arg Asn		Val Leu Tyr Thr Leu		
	Glu Gln Arg Asn His		Leu Tyr Thr Leu Arg		
	Gln Arg Asn His Leu		Tyr Thr Leu Arg Ala		
	Arg Asn His Leu Gln		Thr Leu Arg Ala Pro		
	Asn His Leu Gln Gly		Leu Arg Ala Pro Arg		
	His Leu Gln Gly Lys		Arg Ala Pro Arg Ser		
	Leu Gln Gly Lys Leu		Ala Pro Arg Ser Pro		
	Gln Gly Lys Leu Ser		Pro Arg Ser Pro Lys		

When myosin light chain 2 (SEQ ID NO:8) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of myosin light chain 2 (SEQ ID NO:8). Table 9 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from myosin light chain 2 (SEQ ID NO: 8). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 9, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:8, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 9:

	Ala Pro Lys Lys Ala		Thr Phe Ala Ala Leu		Ala Phe Lys Val Phe
	Pro Lys Lys Ala Lys		Phe Ala Ala Leu Gly		Phe Lys Val Phe Asp
	Lys Lys Ala Lys Lys		Ala Ala Leu Gly Arg		Lys Val Phe Asp Pro
	Lys Ala Lys Lys Arg		Ala Leu Gly Arg Val		Val Phe Asp Pro Glu
	Ala Lys Lys Arg Ala		Leu Gly Arg Val Asn		Phe Asp Pro Glu Gly
	Lys Lys Arg Ala Gly		Gly Arg Val Asn Val		Asp Pro Glu Gly Lys
	Lys Arg Ala Gly Gly		Arg Val Asn Val Lys		Pro Glu Gly Lys Gly
	Arg Ala Gly Gly Ala		Val Asn Val Lys Asn		Glu Gly Lys Gly Val

	Ala Gly Gly Ala Asn		Asn Val Lys Asn Glu		Gly Lys Gly Val Leu
	Gly Gly Ala Asn Ser		Val Lys Asn Glu Glu		Lys Gly Val Leu Lys
	Gly Ala Asn Ser Asn		Lys Asn Glu Glu Ile		Gly Val Leu Lys Ala
	Ala Asn Ser Asn Val		Asn Glu Glu Ile Asp		Val Leu Lys Ala Asp
	Asn Ser Asn Val Phe		Glu Glu Ile Asp Glu		Leu Lys Ala Asp Tyr
	Ser Asn Val Phe Ser		Glu Ile Asp Glu Met		Lys Ala Asp Tyr Val
	Asn Val Phe Ser Met		Ile Asp Glu Met Ile		Ala Asp Tyr Val Arg
	Val Phe Ser Met Phe		Asp Glu Met Ile Lys		Asp Tyr Val Arg Glu
	Phe Ser Met Phe Glu		Glu Met Ile Lys Glu		Tyr Val Arg Glu Met
	Ser Met Phe Glu Gln		Met Ile Lys Glu Ala		Val Arg Glu Met Leu
	Met Phe Glu Gln Thr		Ile Lys Glu Ala Pro		Arg Glu Met Leu Thr
	Phe Glu Gln Thr Gln		Lys Glu Ala Pro Gly		Glu Met Leu Thr Thr
	Glu Gln Thr Gln Ile		Glu Ala Pro Gly Pro		Met Leu Thr Thr Gln
	Gln Thr Gln Ile Gln		Ala Pro Gly Pro Ile		Leu Thr Thr Gln Ala
	Thr Gln Ile Gln Glu		Pro Gly Pro Ile Asn		Thr Thr Gln Ala Glu
	Gln Ile Gln Glu Phe		Gly Pro Ile Asn Phe		Thr Gln Ala Glu Arg
	Ile Gln Glu Phe Lys		Pro Ile Asn Phe Thr		Gln Ala Glu Arg Phe
	Gln Glu Phe Lys Glu		Ile Asn Phe Thr Val		Ala Glu Arg Phe Ser
	Glu Phe Lys Glu Ala		Asn Phe Thr Val Phe		Glu Arg Phe Ser Lys
	Phe Lys Glu Ala Phe		Phe Thr Val Phe Leu		Arg Phe Ser Lys Glu
	Lys Glu Ala Phe Thr		Thr Val Phe Leu Thr		Phe Ser Lys Glu Glu
	Glu Ala Phe Thr Ile		Val Phe Leu Thr Met		Ser Lys Glu Glu Val
	Ala Phe Thr Ile Met		Phe Leu Thr Met Phe		Lys Glu Glu Val Asp
	Phe Thr Ile Met Asp		Leu Thr Met Phe Gly		Glu Glu Val Asp Gln
	Thr Ile Met Asp Gln		Thr Met Phe Gly Glu		Glu Val Asp Gln Met
	Ile Met Asp Gln Asn		Met Phe Gly Glu Lys		Val Asp Gln Met Phe
	Met Asp Gln Asn Arg		Phe Gly Glu Lys Leu		Asp Gln Met Phe Ala
	Asp Gln Asn Arg Asp		Gly Glu Lys Leu Lys		Gln Met Phe Ala Ala
	Gln Asn Arg Asp Gly		Glu Lys Leu Lys Gly		Met Phe Ala Ala Phe
	Asn Arg Asp Gly Phe		Lys Leu Lys Gly Ala		Phe Ala Ala Phe Pro
	Arg Asp Gly Phe Ile		Leu Lys Gly Ala Asp		Ala Ala Phe Pro Pro
	Asp Gly Phe Ile Asp		Lys Gly Ala Asp Pro		Ala Phe Pro Pro Asp

	Gly Phe Ile Asp Lys		Gly Ala Asp Pro Glu		Phe Pro Pro Asp Val
	Phe Ile Asp Lys Asn		Ala Asp Pro Glu Glu		Pro Pro Asp Val Thr
	Ile Asp Lys Asn Asp		Asp Pro Glu Glu Thr		Pro Asp Val Thr Gly
	Asp Lys Asn Asp Leu		Pro Glu Glu Thr Ile		Asp Val Thr Gly Asn
	Lys Asn Asp Leu Arg		Glu Glu Thr Ile Leu		Val Thr Gly Asn Leu
	Asn Asp Leu Arg Asp		Glu Thr Ile Leu Asn		Thr Gly Asn Leu Asp
	Asp Leu Arg Asp Thr		Thr Ile Leu Asn Ala		Gly Asn Leu Asp Tyr
	Leu Arg Asp Thr Phe		Ile Leu Asn Ala Phe		Asn Leu Asp Tyr Lys
	Arg Asp Thr Phe Ala		Leu Asn Ala Phe Lys		Leu Asp Tyr Lys Asn
	Asp Thr Phe Ala Ala		Asn Ala Phe Lys Val		Asp Tyr Lys Asn Leu
	Tyr Lys Asn Leu Val				
	Lys Asn Leu Val His				
	Asn Leu Val His Ile				
	Leu Val His Ile Ile				
	Val His Ile Ile Thr				
	His Ile Ile Thr His				
	Ile Ile Thr His Gly				
	Ile Thr His Gly Glu				
	Thr His Gly Glu Glu				
	His Gly Glu Glu Lys				
	Gly Glu Glu Lys Asp				

When myosin-6 (SEQ ID NO:9) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of myosin-6 (SEQ ID NO:9). Table 10 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from myosin-6 (SEQ ID NO: 9). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 10, plus up to a total of 10 additional consecutive amino acid residues from SEQ ID NO:9, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 10:

	Thr Asp Ala Gln Met		Leu Ser Arg Glu Gly		Leu Phe Asn Leu Lys
	Asp Ala Gln Met Ala		Ser Arg Glu Gly Gly		Phe Asn Leu Lys Glu
	Ala Gln Met Ala Asp		Arg Glu Gly Gly Lys		Asn Leu Lys Glu Arg
	Gln Met Ala Asp Phe		Glu Gly Gly Lys Val		Leu Lys Glu Arg Tyr
	Met Ala Asp Phe Gly		Gly Gly Lys Val Ile		Lys Glu Arg Tyr Ala
	Ala Asp Phe Gly Ala		Gly Lys Val Ile Ala		Glu Arg Tyr Ala Ala
	Asp Phe Gly Ala Ala		Lys Val Ile Ala Glu		Arg Tyr Ala Ala Trp
	Phe Gly Ala Ala Ala		Val Ile Ala Glu Thr		Tyr Ala Ala Trp Met
	Gly Ala Ala Ala Gln		Ile Ala Glu Thr Glu		Ala Ala Trp Met Ile
	Ala Ala Ala Gln Tyr		Ala Glu Thr Glu Asn		Ala Trp Met Ile Tyr
	Ala Ala Gln Tyr Leu		Glu Thr Glu Asn Gly		Trp Met Ile Tyr Thr
	Ala Gln Tyr Leu Arg		Thr Glu Asn Gly Lys		Met Ile Tyr Thr Tyr
	Gln Tyr Leu Arg Lys		Glu Asn Gly Lys Thr		Ile Tyr Thr Tyr Ser
	Tyr Leu Arg Lys Ser		Asn Gly Lys Thr Val		Tyr Thr Tyr Ser Gly
	Leu Arg Lys Ser Glu		Gly Lys Thr Val Thr		Thr Tyr Ser Gly Leu
	Arg Lys Ser Glu Lys		Lys Thr Val Thr Val		Tyr Ser Gly Leu Phe
	Lys Ser Glu Lys Glu		Thr Val Thr Val Lys		Ser Gly Leu Phe Cys
	Ser Glu Lys Glu Arg		Val Thr Val Lys Glu		Gly Leu Phe Cys Val
	Glu Lys Glu Arg Leu		Thr Val Lys Glu Asp		Leu Phe Cys Val Thr
	Lys Glu Arg Leu Glu		Val Lys Glu Asp Gln		Phe Cys Val Thr Val
	Glu Arg Leu Glu Ala		Lys Glu Asp Gln Val		Cys Val Thr Val Asn
	Arg Leu Glu Ala Gln		Glu Asp Gln Val Leu		Val Thr Val Asn Pro
	Leu Glu Ala Gln Thr		Asp Gln Val Leu Gln		Thr Val Asn Pro Tyr
	Glu Ala Gln Thr Arg		Gln Val Leu Gln Gln		Val Asn Pro Tyr Lys
	Ala Gln Thr Arg Pro		Val Leu Gln Gln Asn		Asn Pro Tyr Lys Trp
	Gln Thr Arg Pro Phe		Leu Gln Gln Asn Pro		Pro Tyr Lys Trp Leu
	Thr Arg Pro Phe Asp		Gln Gln Asn Pro Pro		Tyr Lys Trp Leu Pro
	Arg Pro Phe Asp Ile		Gln Asn Pro Pro Lys		Lys Trp Leu Pro Val
	Pro Phe Asp Ile Arg		Asn Pro Pro Lys Phe		Trp Leu Pro Val Tyr
	Phe Asp Ile Arg Thr		Pro Pro Lys Phe Asp		Leu Pro Val Tyr Asn
	Asp Ile Arg Thr Glu		Pro Lys Phe Asp Lys		Pro Val Tyr Asn Ala
	Ile Arg Thr Glu Cys		Lys Phe Asp Lys Ile		Val Tyr Asn Ala Glu

	Arg Thr Glu Cys Phe		Phe Asp Lys Ile Gln		Tyr Asn Ala Glu Val
	Thr Glu Cys Phe Val		Asp Lys Ile Gln Asp		Asn Ala Glu Val Val
	Glu Cys Phe Val Pro		Lys Ile Gln Asp Met		Ala Glu Val Val Ala
	Cys Phe Val Pro Asp		Ile Gln Asp Met Ala		Glu Val Val Ala Ala
	Phe Val Pro Asp Asp		Gln Asp Met Ala Met		Val Val Ala Ala Tyr
	Val Pro Asp Asp Lys		Asp Met Ala Met Leu		Val Ala Ala Tyr Arg
	Pro Asp Asp Lys Glu		Met Ala Met Leu Thr		Ala Ala Tyr Arg Gly
	Asp Asp Lys Glu Glu		Ala Met Leu Thr Phe		Ala Tyr Arg Gly Lys
	Asp Lys Glu Glu Phe		Met Leu Thr Phe Leu		Tyr Arg Gly Lys Lys
	Lys Glu Glu Phe Val		Leu Thr Phe Leu His		Arg Gly Lys Lys Arg
	Glu Glu Phe Val Lys		Thr Phe Leu His Glu		Gly Lys Lys Arg Ser
	Glu Phe Val Lys Ala		Phe Leu His Glu Pro		Lys Lys Arg Ser Glu
	Phe Val Lys Ala Lys		Leu His Glu Pro Ala		Lys Arg Ser Glu Ala
	Val Lys Ala Lys Ile		His Glu Pro Ala Val		Arg Ser Glu Ala Pro
	Lys Ala Lys Ile Leu		Glu Pro Ala Val Leu		Ser Glu Ala Pro Pro
	Ala Lys Ile Leu Ser		Pro Ala Val Leu Phe		Glu Ala Pro Pro His
	Lys Ile Leu Ser Arg		Ala Val Leu Phe Asn		Ala Pro Pro His Ile
	Ile Leu Ser Arg Glu		Val Leu Phe Asn Leu		Pro Pro His Ile Phe
	Pro His Ile Phe Ser		Arg Gly Lys Lys Asp		Thr Gly Lys Leu Ala
	His Ile Phe Ser Ile		Gly Lys Lys Asp Asn		Gly Lys Leu Ala Ser
	Ile Phe Ser Ile Ser		Lys Lys Asp Asn Ala		Lys Leu Ala Ser Ala
	Phe Ser Ile Ser Asp		Lys Asp Asn Ala Asn		Leu Ala Ser Ala Asp
	Ser Ile Ser Asp Asn		Asp Asn Ala Asn Ala		Ala Ser Ala Asp Ile
	Ile Ser Asp Asn Ala		Asn Ala Asn Ala Asn		Ser Ala Asp Ile Glu
	Ser Asp Asn Ala Tyr		Ala Asn Ala Asn Lys		Ala Asp Ile Glu Thr
	Asp Asn Ala Tyr Gln		Asn Ala Asn Lys Gly		Asp Ile Glu Thr Tyr
	Asn Ala Tyr Gln Tyr		Ala Asn Lys Gly Thr		Ile Glu Thr Tyr Leu
	Ala Tyr Gln Tyr Met		Asn Lys Gly Thr Leu		Glu Thr Tyr Leu Leu
	Tyr Gln Tyr Met Leu		Lys Gly Thr Leu Glu		Thr Tyr Leu Leu Glu
	Gln Tyr Met Leu Thr		Gly Thr Leu Glu Asp		Tyr Leu Leu Glu Lys
	Tyr Met Leu Thr Asp		Thr Leu Glu Asp Gln		Leu Leu Glu Lys Ser
	Met Leu Thr Asp Arg		Leu Glu Asp Gln Ile		Leu Glu Lys Ser Arg

	Leu Thr Asp Arg Glu		Glu Asp Gln Ile Ile		Glu Lys Ser Arg Val
	Thr Asp Arg Glu Asn		Asp Gln Ile Ile Gln		Lys Ser Arg Val Ile
	Asp Arg Glu Asn Gln		Gln Ile Ile Gln Ala		Ser Arg Val Ile Phe
	Arg Glu Asn Gln Ser		Ile Ile Gln Ala Asn		Arg Val Ile Phe Gln
	Glu Asn Gln Ser Ile		Ile Gln Ala Asn Pro		Val Ile Phe Gln Leu
	Asn Gln Ser Ile Leu		Gln Ala Asn Pro Ala		Ile Phe Gln Leu Lys
	Gln Ser Ile Leu Ile		Ala Asn Pro Ala Leu		Phe Gln Leu Lys Ala
	Ser Ile Leu Ile Thr		Asn Pro Ala Leu Glu		Gln Leu Lys Ala Glu
	Ile Leu Ile Thr Gly		Pro Ala Leu Glu Ala		Leu Lys Ala Glu Arg
	Leu Ile Thr Gly Glu		Ala Leu Glu Ala Phe		Lys Ala Glu Arg Asn
	Ile Thr Gly Glu Ser		Leu Glu Ala Phe Gly		Ala Glu Arg Asn Tyr
	Thr Gly Glu Ser Gly		Glu Ala Phe Gly Asn		Glu Arg Asn Tyr His
	Gly Glu Ser Gly Ala		Ala Phe Gly Asn Ala		Arg Asn Tyr His Ile
	Glu Ser Gly Ala Gly		Phe Gly Asn Ala Lys		Asn Tyr His Ile Phe
	Ser Gly Ala Gly Lys		Gly Asn Ala Lys Thr		Tyr His Ile Phe Tyr
	Gly Ala Gly Lys Thr		Asn Ala Lys Thr Val		His Ile Phe Tyr Gln
	Ala Gly Lys Thr Val		Ala Lys Thr Val Arg		Ile Phe Tyr Gln Ile
	Gly Lys Thr Val Asn		Lys Thr Val Arg Asn		Phe Tyr Gln Ile Leu
	Lys Thr Val Asn Thr		Thr Val Arg Asn Asp		Tyr Gln Ile Leu Ser
	Thr Val Asn Thr Lys		Val Arg Asn Asp Asn		Gln Ile Leu Ser Asn
	Val Asn Thr Lys Arg		Arg Asn Asp Asn Ser		Ile Leu Ser Asn Lys
	Asn Thr Lys Arg Val		Asn Asp Asn Ser Ser		Leu Ser Asn Lys Lys
	Thr Lys Arg Val Ile		Asp Asn Ser Ser Arg		Ser Asn Lys Lys Pro
	Lys Arg Val Ile Gln		Asn Ser Ser Arg Phe		Asn Lys Lys Pro Glu
	Arg Val Ile Gln Tyr		Ser Ser Arg Phe Gly		Lys Lys Pro Glu Leu
	Val Ile Gln Tyr Phe		Ser Arg Phe Gly Lys		Lys Pro Glu Leu Leu
	Ile Gln Tyr Phe Ala		Arg Phe Gly Lys Phe		Pro Glu Leu Leu Asp
	Gln Tyr Phe Ala Ser		Phe Gly Lys Phe Ile		Glu Leu Leu Asp Met
	Tyr Phe Ala Ser Ile		Gly Lys Phe Ile Arg		Leu Leu Asp Met Leu
	Phe Ala Ser Ile Ala		Lys Phe Ile Arg Ile		Leu Asp Met Leu Leu
	Ala Ser Ile Ala Ala		Phe Ile Arg Ile His		Asp Met Leu Leu Val
	Ser Ile Ala Ala Ile		Ile Arg Ile His Phe		Met Leu Leu Val Thr

	Ile Ala Ala Ile Gly		Arg Ile His Phe Gly		Leu Leu Val Thr Asn
	Ala Ala Ile Gly Asp		Ile His Phe Gly Ala		Leu Val Thr Asn Asn
	Ala Ile Gly Asp Arg		His Phe Gly Ala Thr		Val Thr Asn Asn Pro
	Ile Gly Asp Arg Gly		Phe Gly Ala Thr Gly		Thr Asn Asn Pro Tyr
	Gly Asp Arg Gly Lys		Gly Ala Thr Gly Lys		Asn Asn Pro Tyr Asp
	Asp Arg Gly Lys Lys		Ala Thr Gly Lys Leu		Asn Pro Tyr Asp Tyr
	Pro Tyr Asp Tyr Ala		Tyr Gly Asn Met Lys		Val Thr Lys Gly Gln
	Tyr Asp Tyr Ala Phe		Gly Asn Met Lys Phe		Thr Lys Gly Gln Ser
	Asp Tyr Ala Phe Val		Asn Met Lys Phe Lys		Lys Gly Gln Ser Val
	Tyr Ala Phe Val Ser		Met Lys Phe Lys Gln		Gly Gln Ser Val Gln
	Ala Phe Val Ser Gln		Lys Phe Lys Gln Lys		Gln Ser Val Gln Gln
	Phe Val Ser Gln Gly		Phe Lys Gln Lys Gln		Ser Val Gln Gln Val
	Val Ser Gln Gly Glu		Lys Gln Lys Gln Arg		Val Gln Gln Val Tyr
	Ser Gln Gly Glu Val		Gln Lys Gln Arg Glu		Gln Gln Val Tyr Tyr
	Gln Gly Glu Val Ser		Lys Gln Arg Glu Glu		Gln Val Tyr Tyr Ser
	Gly Glu Val Ser Val		Gln Arg Glu Glu Gln		Val Tyr Tyr Ser Ile
	Glu Val Ser Val Ala		Arg Glu Glu Gln Ala		Tyr Tyr Ser Ile Gly
	Val Ser Val Ala Ser		Glu Glu Gln Ala Glu		Tyr Ser Ile Gly Ala
	Ser Val Ala Ser Ile		Glu Gln Ala Glu Pro		Ser Ile Gly Ala Leu
	Val Ala Ser Ile Asp		Gln Ala Glu Pro Asp		Ile Gly Ala Leu Ala
	Ala Ser Ile Asp Asp		Ala Glu Pro Asp Gly		Gly Ala Leu Ala Lys
	Ser Ile Asp Asp Ser		Glu Pro Asp Gly Thr		Ala Leu Ala Lys Ala
	Ile Asp Asp Ser Glu		Pro Asp Gly Thr Glu		Leu Ala Lys Ala Val
	Asp Asp Ser Glu Glu		Asp Gly Thr Glu Asp		Ala Lys Ala Val Tyr
	Asp Ser Glu Glu Leu		Gly Thr Glu Asp Ala		
	Ser Glu Glu Leu Met		Thr Glu Asp Ala Asp		
	Glu Glu Leu Met Ala		Glu Asp Ala Asp Lys		
	Glu Leu Met Ala Thr		Asp Ala Asp Lys Ser		
	Leu Met Ala Thr Asp		Ala Asp Lys Ser Ala		
	Met Ala Thr Asp Ser		Asp Lys Ser Ala Tyr		
	Ala Thr Asp Ser Ala		Lys Ser Ala Tyr Leu		
	Thr Asp Ser Ala Phe		Ser Ala Tyr Leu Met		

	Asp Ser Ala Phe Asp		Ala Tyr Leu Met Gly		
	Ser Ala Phe Asp Val		Tyr Leu Met Gly Leu		
	Ala Phe Asp Val Leu		Leu Met Gly Leu Asn		
	Phe Asp Val Leu Gly		Met Gly Leu Asn Ser		
	Asp Val Leu Gly Phe		Gly Leu Asn Ser Ala		
	Val Leu Gly Phe Thr		Leu Asn Ser Ala Asp		
	Leu Gly Phe Thr Ser		Asn Ser Ala Asp Leu		
	Gly Phe Thr Ser Glu		Ser Ala Asp Leu Leu		
	Phe Thr Ser Glu Glu		Ala Asp Leu Leu Lys		
	Thr Ser Glu Glu Lys		Asp Leu Leu Lys Gly		
	Ser Glu Glu Lys Ala		Leu Leu Lys Gly Leu		
	Glu Glu Lys Ala Gly		Leu Lys Gly Leu Cys		
	Glu Lys Ala Gly Val		Lys Gly Leu Cys His		
	Lys Ala Gly Val Tyr		Gly Leu Cys His Pro		
	Ala Gly Val Tyr Lys		Leu Cys His Pro Arg		
	Gly Val Tyr Lys Leu		Cys His Pro Arg Val		
	Val Tyr Lys Leu Thr		His Pro Arg Val Lys		
	Tyr Lys Leu Thr Gly		Pro Arg Val Lys Val		
	Lys Leu Thr Gly Ala		Arg Val Lys Val Gly		
	Leu Thr Gly Ala Ile		Val Lys Val Gly Asn		
	Thr Gly Ala Ile Met		Lys Val Gly Asn Glu		
	Gly Ala Ile Met His		Val Gly Asn Glu Tyr		
	Ala Ile Met His Tyr		Gly Asn Glu Tyr Val		
	Ile Met His Tyr Gly		Asn Glu Tyr Val Thr		
	Met His Tyr Gly Asn		Glu Tyr Val Thr Lys		
	His Tyr Gly Asn Met		Tyr Val Thr Lys Gly		

When myosin-7 (SEQ ID NO:10) is the target self-antigen, the peptide reagent comprises any sequence of 5 to 15 consecutive amino acid residues from anywhere in the amino acid sequence of myosin-7 (SEQ ID NO:10). Table 11 lists amino acid sequences for exemplary peptide reagents consisting of 5 consecutive amino acid residues from myosin-7 (SEQ ID NO: 10). Additional peptide reagents may have a length of up to 15 amino acid residues, comprising any one of the listed 5-amino acid long sequences in Table 11, plus up

to a total of 10 additional consecutive amino acid residues from SEQ ID NO:10, that are continuous (from either side within the protein amino sequence) with the 5-amino acid long sequence.

TABLE 11:

	Gly Asp Ser Glu Met		Val Ser Arg Glu Gly		Leu Tyr Asn Leu Lys
	Asp Ser Glu Met Ala		Ser Arg Glu Gly Gly		Tyr Asn Leu Lys Asp
	Ser Glu Met Ala Val		Arg Glu Gly Gly Lys		Asn Leu Lys Asp Arg
	Glu Met Ala Val Phe		Glu Gly Gly Lys Val		Leu Lys Asp Arg Tyr
	Met Ala Val Phe Gly		Gly Gly Lys Val Thr		Lys Asp Arg Tyr Gly
	Ala Val Phe Gly Ala		Gly Lys Val Thr Ala		Asp Arg Tyr Gly Ser
	Val Phe Gly Ala Ala		Lys Val Thr Ala Glu		Arg Tyr Gly Ser Trp
	Phe Gly Ala Ala Ala		Val Thr Ala Glu Thr		Tyr Gly Ser Trp Met
	Gly Ala Ala Ala Pro		Thr Ala Glu Thr Glu		Gly Ser Trp Met Ile
	Ala Ala Ala Pro Tyr		Ala Glu Thr Glu Tyr		Ser Trp Met Ile Tyr
	Ala Ala Pro Tyr Leu		Glu Thr Glu Tyr Gly		Trp Met Ile Tyr Thr
	Ala Pro Tyr Leu Arg		Thr Glu Tyr Gly Lys		Met Ile Tyr Thr Tyr
	Pro Tyr Leu Arg Lys		Glu Tyr Gly Lys Thr		Ile Tyr Thr Tyr Ser
	Tyr Leu Arg Lys Ser		Tyr Gly Lys Thr Val		Tyr Thr Tyr Ser Gly
	Leu Arg Lys Ser Glu		Gly Lys Thr Val Thr		Thr Tyr Ser Gly Leu
	Arg Lys Ser Glu Lys		Lys Thr Val Thr Val		Tyr Ser Gly Leu Phe
	Lys Ser Glu Lys Glu		Thr Val Thr Val Lys		Ser Gly Leu Phe Cys
	Ser Glu Lys Glu Arg		Val Thr Val Lys Glu		Gly Leu Phe Cys Val
	Glu Lys Glu Arg Leu		Thr Val Lys Glu Asp		Leu Phe Cys Val Thr
	Lys Glu Arg Leu Glu		Val Lys Glu Asp Gln		Phe Cys Val Thr Val
	Glu Arg Leu Glu Ala		Lys Glu Asp Gln Val		Cys Val Thr Val Asn
	Arg Leu Glu Ala Gln		Glu Asp Gln Val Met		Val Thr Val Asn Pro
	Leu Glu Ala Gln Thr		Asp Gln Val Met Gln		Thr Val Asn Pro Tyr
	Glu Ala Gln Thr Arg		Gln Val Met Gln Gln		Val Asn Pro Tyr Lys
	Ala Gln Thr Arg Pro		Val Met Gln Gln Asn		Asn Pro Tyr Lys Trp
	Gln Thr Arg Pro Phe		Met Gln Gln Asn Pro		Pro Tyr Lys Trp Leu
	Thr Arg Pro Phe Asp		Gln Gln Asn Pro Pro		Tyr Lys Trp Leu Pro
	Arg Pro Phe Asp Leu		Gln Asn Pro Pro Lys		Lys Trp Leu Pro Val

	Pro Phe Asp Leu Lys		Asn Pro Pro Lys Phe		Trp Leu Pro Val Tyr
	Phe Asp Leu Lys Lys		Pro Pro Lys Phe Asp		Leu Pro Val Tyr Thr
	Asp Leu Lys Lys Asp		Pro Lys Phe Asp Lys		Pro Val Tyr Thr Pro
	Leu Lys Lys Asp Val		Lys Phe Asp Lys Ile		Val Tyr Thr Pro Glu
	Lys Lys Asp Val Phe		Phe Asp Lys Ile Glu		Tyr Thr Pro Glu Val
	Lys Asp Val Phe Val		Asp Lys Ile Glu Asp		Thr Pro Glu Val Val
	Asp Val Phe Val Pro		Lys Ile Glu Asp Met		Pro Glu Val Val Ala
	Val Phe Val Pro Asp		Ile Glu Asp Met Ala		Glu Val Val Ala Ala
	Phe Val Pro Asp Asp		Glu Asp Met Ala Met		Val Val Ala Ala Tyr
	Val Pro Asp Asp Lys		Asp Met Ala Met Leu		Val Ala Ala Tyr Arg
	Pro Asp Asp Lys Gln		Met Ala Met Leu Thr		Ala Ala Tyr Arg Gly
	Asp Asp Lys Gln Glu		Ala Met Leu Thr Phe		Ala Tyr Arg Gly Lys
	Asp Lys Gln Glu Phe		Met Leu Thr Phe Leu		Tyr Arg Gly Lys Lys
	Lys Gln Glu Phe Val		Leu Thr Phe Leu His		Arg Gly Lys Lys Arg
	Gln Glu Phe Val Lys		Thr Phe Leu His Glu		Gly Lys Lys Arg Ser
	Glu Phe Val Lys Ala		Phe Leu His Glu Pro		Lys Lys Arg Ser Glu
	Phe Val Lys Ala Lys		Leu His Glu Pro Ala		Lys Arg Ser Glu Ala
	Val Lys Ala Lys Ile		His Glu Pro Ala Val		Arg Ser Glu Ala Pro
	Lys Ala Lys Ile Val		Glu Pro Ala Val Leu		Ser Glu Ala Pro Pro
	Ala Lys Ile Val Ser		Pro Ala Val Leu Tyr		Glu Ala Pro Pro His
	Lys Ile Val Ser Arg		Ala Val Leu Tyr Asn		Ala Pro Pro His Ile
	Ile Val Ser Arg Glu		Val Leu Tyr Asn Leu		Pro Pro His Ile Phe
	Pro His Ile Phe Ser		Arg Ser Lys Lys Asp		Gly Lys Leu Ala Ser
	His Ile Phe Ser Ile		Ser Lys Lys Asp Gln		Lys Leu Ala Ser Ala
	Ile Phe Ser Ile Ser		Lys Lys Asp Gln Ser		Leu Ala Ser Ala Asp
	Phe Ser Ile Ser Asp		Lys Asp Gln Ser Pro		Ala Ser Ala Asp Ile
	Ser Ile Ser Asp Asn		Asp Gln Ser Pro Gly		Ser Ala Asp Ile Glu
	Ile Ser Asp Asn Ala		Gln Ser Pro Gly Lys		Ala Asp Ile Glu Thr
	Ser Asp Asn Ala Tyr		Ser Pro Gly Lys Gly		Asp Ile Glu Thr Tyr
	Asp Asn Ala Tyr Gln		Pro Gly Lys Gly Thr		Ile Glu Thr Tyr Leu
	Asn Ala Tyr Gln Tyr		Gly Lys Gly Thr Leu		Glu Thr Tyr Leu Leu
	Ala Tyr Gln Tyr Met		Lys Gly Thr Leu Glu		Thr Tyr Leu Leu Glu

	Tyr Gln Tyr Met Leu		Gly Thr Leu Glu Asp		Tyr Leu Leu Glu Lys
	Gln Tyr Met Leu Thr		Thr Leu Glu Asp Gln		Leu Leu Glu Lys Ser
	Tyr Met Leu Thr Asp		Leu Glu Asp Gln Ile		Leu Glu Lys Ser Arg
	Met Leu Thr Asp Arg		Glu Asp Gln Ile Ile		Glu Lys Ser Arg Val
	Leu Thr Asp Arg Glu		Asp Gln Ile Ile Gln		Lys Ser Arg Val Ile
	Thr Asp Arg Glu Asn		Gln Ile Ile Gln Ala		Ser Arg Val Ile Phe
	Asp Arg Glu Asn Gln		Ile Ile Gln Ala Asn		Arg Val Ile Phe Gln
	Arg Glu Asn Gln Ser		Ile Gln Ala Asn Pro		Val Ile Phe Gln Leu
	Glu Asn Gln Ser Ile		Gln Ala Asn Pro Ala		Ile Phe Gln Leu Lys
	Asn Gln Ser Ile Leu		Ala Asn Pro Ala Leu		Phe Gln Leu Lys Ala
	Gln Ser Ile Leu Ile		Asn Pro Ala Leu Glu		Gln Leu Lys Ala Glu
	Ser Ile Leu Ile Thr		Pro Ala Leu Glu Ala		Leu Lys Ala Glu Arg
	Ile Leu Ile Thr Gly		Ala Leu Glu Ala Phe		Lys Ala Glu Arg Asp
	Leu Ile Thr Gly Glu		Leu Glu Ala Phe Gly		Ala Glu Arg Asp Tyr
	Ile Thr Gly Glu Ser		Glu Ala Phe Gly Asn		Glu Arg Asp Tyr His
	Thr Gly Glu Ser Gly		Ala Phe Gly Asn Ala		Arg Asp Tyr His Ile
	Gly Glu Ser Gly Ala		Phe Gly Asn Ala Lys		Asp Tyr His Ile Phe
	Glu Ser Gly Ala Gly		Gly Asn Ala Lys Thr		Tyr His Ile Phe Tyr
	Ser Gly Ala Gly Lys		Asn Ala Lys Thr Val		His Ile Phe Tyr Gln
	Gly Ala Gly Lys Thr		Ala Lys Thr Val Arg		Ile Phe Tyr Gln Ile
	Ala Gly Lys Thr Val		Lys Thr Val Arg Asn		
	Gly Lys Thr Val Asn		Thr Val Arg Asn Asp		
	Lys Thr Val Asn Thr		Val Arg Asn Asp Asn		
	Thr Val Asn Thr Lys		Arg Asn Asp Asn Ser		
	Val Asn Thr Lys Arg		Asn Asp Asn Ser Ser		
	Asn Thr Lys Arg Val		Asp Asn Ser Ser Arg		
	Thr Lys Arg Val Ile		Asn Ser Ser Arg Phe		
	Lys Arg Val Ile Gln		Ser Ser Arg Phe Gly		
	Arg Val Ile Gln Tyr		Ser Arg Phe Gly Lys		
	Val Ile Gln Tyr Phe		Arg Phe Gly Lys Phe		
	Ile Gln Tyr Phe Ala		Phe Gly Lys Phe Ile		
	Gln Tyr Phe Ala Val		Gly Lys Phe Ile Arg		

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

Any one of the peptide reagents optionally can be modified at either or both of the N-terminal and C-terminal ends. N-terminal modifications include for example: acetylation [Ac], benzyloxycarbonyl [Cbz], biotin [Btn], cinnamoylation [Cinn], dabcyl [Dabc], dabsyl [Dabs], innamoylation [Cinn], dabcyl [Dabc], dabsyl [Dabs], dansyl [Dans], dinitrophenyl [Dnp], fluorescein [Flc], FMOC [Fmoc], formylation [Form], lissamine rhodamine [Liss], myristoylation [Myrs], N-methyl [Nme], palmitoylation [Palm], steroylation [Ster], and 7-methoxycoumarin acetic acid[Mca]. C-terminal modifications include for example: amide [NH2], 4-Branch MAP resin [MAPC], and hydroxyl [OH].

Given a protein and thus a starting amino acid sequence from which a peptide reagent is to be derived, the peptide, or a library of multiple peptides, including peptides with modifications to either or both terminal ends, can be prepared by readily commercially accessible custom peptide synthesis services. Such services are now routinely available from, for example Sigma-Genosys (as PEPscreen®), Invitrogen and GeneTel Laboatories.

Peptide reagents according to the present disclosure can be tested for inhibition of autoantibody binding to the target protein by any of several detection methods as will be recognized by those of skill in the art. Typically a peptide reagent is prepared in a diluent to produce several solutions of varying concentrations. Each solution is combined with a selected amount of a test sample containing a known amount of autoantibody and target protein. A detection conjugate that includes a detectable label and a specific binding partner, i.e. antibody, against the target protein is also added. A signal generated by the detection conjugate can be used to quantify the relative inhibitory activity of each dilution of the peptide reagent with respect to autoantibody binding to the target protein.

For example, equimolar starting solutions of each peptide reagent, each having a different amino acid sequence derived from the target protein, can be obtained and then diluted in a suitable pre-incubation diluent to give solutions of pre-selected, varying concentrations, typically in the nmol/mL range. The target protein, typically a recombinant protein, can be coated in a suitable buffer solution on a microplate and maintained under conditions sufficient to obtain binding of the target protein to the plate, for example at 38 °C, for about 1 h. The protein can then be overcoated sequentially with bovine serum albumin and a solution of sucrose in PBS. A detection conjugate can be prepared by labeling a murine anti-human IgG with a detectable label according to labeling methods well-known in the art. For example, the detectable label can be but is not limited to a chemiluminescent compound, such s an acridinium compound.

Each dilution of the inhibitor peptide reagent is then mixed, preferably at about a 1:1 ratio by volume, with a test sample that contains a known amount of endogenous autoantibodies to the target self-antigen. The resulting solutions are arrayed in microplates, sealed and maintained under conditions sufficient to obtain binding of the peptide reagent to the autoantibodies, for example for a period of about 6 to 24 hours at ambient temperature. Test samples that are positive and low controls are diluted with a suitable preincubation diluent and arrayed, for example in triplicate, on the microplate. The plates are incubated under conditions sufficient to obtain binding, for example at 37 °C for at least about 2 hours, and the plate is washed with a suitable buffer such as ARCHITECT® Wash Buffer. A detection conjugate is then added to the plate. For example, a detection conjugate can be a murine anti-human IgG specific monoclonal antibody conjugated to a detectable label. The plate is incubated again under conditions sufficient to achieve binding of the detection conjugate to the target self-antigen, for example at 37 °C for about 1 hour, before a final wash with the wash buffer.

For detection, the microplate is processed according to methods appropriate for the particular label and detection method selected. For example, when using a detection conjugate in which an acridinium compound is the detectable label, the microplate is loaded into a microplate reader (e.g. a Mithras microplate reader, Berthold Technologies Inc, Oak Ridge, TN), and then equilibrated at a suitable temperature, for example at 28 °C. A chemiluminescence signal from each well is recorded for a period of seconds following sequential addition of a pre-trigger solution and a trigger solution. The resulting chemiluminescent signals are then recorded. Data analysis of the signals can include a comparison of the signals as a plot of the ratio of signal to the low control (S/LC) against

concentration of each peptide reagent to reveal the relative strength of inhibition by each peptide reagent.

C. Immunoassay for Detecting a Protein of Interest in a Test Sample

The present disclosure also relates methods of using the peptide reagents as disclosed herein in immunoassays for detecting protein analytes of interest in a test sample in which autoantibodies against the target protein may or may not be present. The protein analytes of interest are typically self-antigens. As set forth elsewhere herein, examples of self-antigens which are proteins for which autoantibodies have been described include but are not limited to cardiac troponin, myeloperoxidase (MPO), prostate specific antigen (PSA), and thyroid stimulating hormone (TSH). It will be understood that the peptide reagents and related methods described herein are also applicable to the detection of any other protein of diagnostic interest for which autoantibodies not yet described may interfere with immunodetection of the protein.

The methods of the present disclosure involves obtaining a test sample from a subject and then detecting the presence of a protein of interest, especially a self-antigen of clinical interest, using immunodetection, while compensating for the presence of any autoantibodies against the analyte that may be present in the sample. This is achieved in part by providing a peptide reagent derived from the protein, which inhibits binding to the protein of the autoantibody that may be present in the sample.

Immunoassay Methods

It will be recognized that methods of the present disclosure can be applied to immunoassays carried out in any of a wide variety of formats. The various immunoassay formats can be applied both to detection per se of a protein of interest, and also to testing of peptide reagents as disclosed herein to evaluate the inhibitory strength of a peptide reagent. A general review of immunoassays is available in METHODS IN CELL BIOLOGY VOLUME 37: ANTIBODIES IN CELL BIOLOGY, Asai, ed. Academic Press, Inc. New York (1993), and BASIC AND CLINICAL IMMUNOLOGY 7TH EDITION, Stites & Terr, eds. (1991), which are herein incorporated by reference in their entirety.

A peptide reagent according to the present disclosure assists in immunodetection of at least one protein (antigen) of interest in a test sample in which autoantibodies to the protein may be present. As described elsewhere herein, the protein from which the peptide reagent is derived can be, for example, selected from the group consisting of: cardiac troponin I, cardiac troponin T, thyroid stimulating hormone (TSH), beta-human chorionic gonadotropin (beta-

HCG), myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (hBNP), myosin light chain 2, myosin-6 and myosin-7. Typically the test sample is for example whole blood, serum or plasma, but can be any biological material, preferably is a biological fluid, suspected of containing a protein of interest and which may also include autoantibodies to the protein of interest.

In use, at least one peptide reagent as disclosed herein is combined with the test sample to form a first mixture. Thus the first mixture contains at least the peptide reagent, and may contain an amount of the target protein and any autoantibodies against the target protein. When the target protein and endogenous autoantibodies against the protein are present in the sample, the peptide reagent disrupts, i.e. blocks the interaction between the autoantibody in the test sample and the protein, leaving the target protein free for specific binding with another binding partner. The method then proceeds according to a typical sandwich immunoassay format. For example, a second mixture is then prepared by combining the first mixture and a first specific binding partner, namely an antibody that binds specifically with the protein of interest. The protein and antibody pair form a first specific binding partner-protein complex. A detection conjugate, i.e. an antibody conjugated to a detectable label, is then introduced to the second mixture. The antibody of the detection conjugate is also a specific binding partner of the protein, i.e. a second specific binding partner. The antibody of the detection conjugate binds to the first specific binding partner-protein complex to form an immunodetection complex that includes the first specific binding partner, protein and second specific binding partner. As the peptide reagent prevents binding of any autoantibody present in the sample to the target protein, the peptide reagent thus prevents autoantibodies from interfering with formation of the immunodetection complex. A signal is generated by or emitted from the detectable label on the detection conjugate, and the signal is used to detect presence of the protein of interest in the test sample. The signal generated by the detection conjugate is proportional to the concentration of the protein of interest as determined by the rate of formation (k1) of the immunodetection complex versus the rate of dissociation of the immunodetection complex (k2).

The method may involve, for example, use of an acridinium compound as the detectable label. When an acridinium compound is used, the method may further include generating or providing a source of hydrogen peroxide to the second mixture, adding a basic solution to the resulting mixture, and measuring the light signal generated or emitted and detecting the protein of interest in the sample. The hydrogen peroxide source may be a

buffer, a solution containing hydrogen peroxide, or a hydrogen peroxide generating enzyme. The basic solution is for example a solution having a pH of at least about 10.

The method can optionally involve use of a solid phase. For example, the first specific binding partner can be immobilized on a solid phase either before or after the formation of the first specific binding partner-protein complex. The second specific binding partner can be immobilized on a solid phase either before or after formation of the first specific binding partner-protein-second specific binding partner complex. The solid phase when used can be any suitable material with sufficient surface affinity to bind the antibodies being used, and can take any of a number of forms, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, a scaffolding molecule, quartz crystal, film, filter paper, disc or a chip. Useful solid phase materials include: natural polymeric carbohydrates and their synthetically modified, crosslinked, or substituted derivatives, such as agar, agarose, cross-linked alginic acid, substituted and cross-linked guar gums, cellulose esters, especially with nitric acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins and derivatives, including cross-linked or modified gelatins; natural hydrocarbon polymers, such as latex and rubber; synthetic polymers, such as vinyl polymers, including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolyzed derivatives, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters, polyamides, and other polymers, such as polyurethanes or polyepoxides; inorganic materials such as sulfates or carbonates of alkaline earth metals and magnesium, including barium sulfate, calcium sulfate, calcium carbonate, silicates of alkali and alkaline earth metals, aluminum and magnesium; and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silica gel, or glass (these materials may be used as filters with the above polymeric materials); and mixtures or copolymers of the above classes, such as graft copolymers obtained by initializing polymerization of synthetic polymers on a pre-existing natural polymer. All of these materials may be used in suitable shapes, such as films, sheets, tubes, particulates, or plates, or they may be coated onto, bonded, or laminated to appropriate inert carriers, such as paper, glass, plastic films, fabrics, or the like. Nitrocellulose has excellent absorption and adsorption qualities for a wide variety of reagents including monoclonal antibodies. Nylon also possesses similar characteristics and also is suitable.

Alternatively, the solid phase can constitute microparticles. Microparticles useful in the present disclosure can be selected by one skilled in the art from any suitable type of

particulate material and include those composed of polystyrene, polymethylacrylate, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate, or similar materials. Further, the microparticles can be magnetic or paramagnetic microparticles, such as carboxylated magnetic microparticles. The methods of the present disclosure can be adapted for use in systems that utilize microparticle technology including automated and semi-automated systems wherein the solid phase comprises a microparticle. Such systems include those described in pending U.S. App. No. 425,651 and U.S. Pat. No. 5,089,424, which correspond to published EPO App. Nos. EP 0 425 633 and EP 0 424 634, respectively, and U.S. Pat. No. 5,006,309.

In particular embodiments, the solid phase includes one or more electrodes. Antibodies can be affixed, directly or indirectly, to the electrode(s). In one embodiment, for example, an antibody of the first specific binding partner can be affixed to magnetic or paramagnetic microparticles, which are then positioned in the vicinity of the electrode surface using a magnet. Systems in which one or more electrodes serve as the solid phase are useful where detection is based on electrochemical interactions. Exemplary systems of this type are described, for example, in U.S. Pat. No. 6,887,714 (issued May 3, 2005). The basic method is described further below with respect to electrochemical detection.

Other considerations affecting the choice of a solid phase include the ability to minimize non-specific binding of labeled entities and compatibility with the labeling system employed. For, example, solid phases used with fluorescent labels should have sufficiently low background fluorescence to allow signal detection.

Thus, according to the present disclosure, an immunoassay of the present disclosure to detect the presence of a protein of interest is a heterogeneous assay employing a solid phase which can be a solid support. The immunoassay can be performed for example by immobilizing an exogenous antibody on the solid phase, wherein the exogenous antibody is reactive with at least one epitope on the protein of interest and functions as the first specific binding partner. The peptide reagent is introduced to the test sample. The test sample is then contacted with first specific binding partner, under conditions sufficient for specific binding of the first specific binding partner to the protein of interest, thus forming a first specific binding partner-protein complex bound to the solid phase. In the case of a test sample containing at least one autoantibody against the protein, the peptide reagent blocks the interaction between the protein of interest and the autoantibody. The first specific binding partner-protein complex bound to the solid phase is contacted with the detection conjugate under conditions sufficient for specific binding of the detection conjugate to any of the

protein of interest that is present in the test sample. An immunodetection complex is thus formed, which includes the first specific binding partner-protein complex and the detection conjugate.

Typically the detection conjugate includes a detectable label. Depending on the detection approach used, an optical, electrical, or change-of-state signal of the immunodetection complex is measured. The immunodetection complex is thus typically a configuration of molecules that once formed generates a signal susceptible to physical detection and/or quantification. Although the immunoassay is described above as including a sequence of steps for illustrative purposes, the test sample may be contacted with the first (capture) antibody and the second (detection) antibody simultaneously or sequentially, in any order. Regardless of the order of contact, if autoantibodies are present in the sample, the peptide reagent blocks interaction of the protein of interest with the autoantibodies that are present in the test sample.

In one format of a sandwich immunoassay according to the present disclosure, detecting comprises detecting a signal from the solid phase-affixed immunodetection complex, which includes the first specific binding partner, protein of interest and second specific binding partner (detection conjugate). In one embodiment, the immunodetection complex is separated from the solid phase, typically by washing, and the signal from the bound label is detected. In another format of a sandwich immunoassay according to the present disclosure, the immunodetection complex remains a solid phase-affixed complex, which is then detected.

Antibodies

In the immunoassays according to the present disclosure, the first specific binding partner can be an antibody including a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a human antibody, an affinity maturated antibody or an antibody fragment. Similarly, the second antibody can be a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a human antibody, an affinity maturated antibody or an antibody fragment.

While monoclonal antibodies are highly specific to the protein/antigen, a polyclonal antibody can preferably be used as the capture (first) antibody to immobilize as much of the protein/antigen as possible. A monoclonal antibody with inherently higher binding specificity for the protein/antigen may then preferably be used as the detection (second) antibody. In any case, the antibody serving as the first specific binding partner and that

serving as the second specific binding partner preferably recognize two non-overlapping epitopes on the protein to avoid blockage of, or interference by one with the epitope recognized by the other. Preferably the antibodies being used are capable of binding simultaneously to different epitopes on the protein of interest, each without interfering with the binding of the other.

Polyclonal antibodies are raised by injecting (e.g., subcutaneous or intramuscular injection) an immunogen into a suitable non-human mammal (e.g., a mouse or a rabbit). Generally, the immunogen should induce production of high titers of antibody with relatively high affinity for the target antigen (protein of interest).

If desired, the antigen may be conjugated to a carrier protein by conjugation techniques that are well known in the art. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The conjugate is then used to immunize the animal.

The antibodies are then obtained from blood samples taken from the animal. The techniques used to produce polyclonal antibodies are extensively described in the literature (see, e.g., Methods of Enzymology, "Production of Antisera With Small Doses of Immunogen: Multiple Intradermal Injections," Langone, et al. eds. (Acad. Press, 1981)). Polyclonal antibodies produced by the animals can be further purified, for example, by binding to and elution from a matrix to which the target antigen is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal, as well as monoclonal, antibodies (see, e.g., Coligan, et al. (1991) Unit 9, Current Protocols in Immunology, Wiley Interscience).

For many applications, monoclonal antibodies (mAbs) are preferred. The general method used for production of hybridomas secreting mAbs is well known (Kohler and Milstein (1975) Nature, 256:495). Briefly, as described by Kohler and Milstein, the technique involves isolating lymphocytes from regional draining lymph nodes of five separate cancer patients with either melanoma, teratocarcinoma or cancer of the cervix, glioma or lung, pooling the cells, and fusing the cells with SHFP-1. Hybridomas are screened for production of antibody that binds to cancer cell lines. Confirmation of specificity among mAbs can be accomplished using routine screening techniques such as ELISA to determine the elementary reaction pattern of the mAb of interest.

As used herein, the term "antibody" encompasses antigen-binding antibody fragments, e.g., single chain antibodies (scFv or others), which can be produced/selected using phage display technology. The ability to express antibody fragments on the surface of viruses that

infect bacteria (bacteriophage or phage) makes it possible to isolate a single binding antibody fragment, e.g., from a library of greater than 10^{10} nonbinding clones. To express antibody fragments on the surface of phage (phage display), an antibody fragment gene is inserted into the gene encoding a phage surface protein (e.g., pIII) and the antibody fragment-pIII fusion protein is displayed on the phage surface (McCafferty et al. (1990) Nature, 348: 552-554; Hoogenboom et al. (1991) Nucleic Acids Res. 19: 4133-4137).

Since the antibody fragments on the surface of the phage are functional, phage-bearing antigen-binding antibody fragments can be separated from non-binding phage by antigen affinity chromatography (McCafferty et al. (1990) Nature, 348: 552-554). Depending on the affinity of the antibody fragment, enrichment factors of 20-fold-1,000,000-fold are obtained for a single round of affinity selection. By infecting bacteria with the eluted phage, however, more phage can be grown and subjected to another round of selection. In this way, an enrichment of 1000-fold in one round can become 1,000,000-fold in two rounds of selection (McCafferty et al. (1990) Nature, 348: 552-554). Thus, even when enrichments are low (Marks et al. (1991) J. Mol. Biol. 222: 581-597), multiple rounds of affinity selection can lead to the isolation of rare phage. Since selection of the phage antibody library on antigen results in enrichment, the majority of clones bind antigen after as few as three to four rounds of selection. Thus only a relatively small number of clones (several hundred) need to be analyzed for binding to antigen.

Human antibodies can be produced without prior immunization by displaying very large and diverse V-gene repertoires on phage (Marks et al. (1991) J. Mol. Biol. 222: 581-597). In one embodiment, natural VH and VL repertoires present in human peripheral blood lymphocytes are isolated from unimmunized donors by PCR. The V-gene repertoires can be spliced together at random using PCR to create a scFv gene repertoire which can be cloned into a phage vector to create a library of 30 million phage antibodies (Id.). From a single "naive" phage antibody library, binding antibody fragments have been isolated against more than 17 different antigens, including haptens, polysaccharides, and proteins (Marks et al. (1991) J. Mol. Biol. 222: 581-597; Marks et al. (1993). Bio/Technology. 10: 779-783; Griffiths et al. (1993) EMBO J. 12: 725-734; Clackson et al. (1991) Nature. 352: 624-628). Antibodies have been produced against self proteins, including human thyroglobulin, immunoglobulin, tumor necrosis factor, and CEA (Griffiths et al. (1993) EMBO J. 12: 725-734). The antibody fragments are highly specific for the antigen used for selection and have affinities in the 1 nM to 100 nM range (Marks et al. (1991) J. Mol. Biol. 222: 581-597;

Griffiths et al. (1993) EMBO J. 12: 725-734). Larger phage antibody libraries result in the isolation of more antibodies of higher binding affinity to a greater proportion of antigens.

As those of skill in the art readily appreciate, antibodies can be prepared by any of a number of commercial services (e.g., Berkeley Antibody Laboratories, Bethyl Laboratories, Anawa, Eurogenetec, etc.).

Detection Systems In General

As discussed above, immunoassays according to the present disclosure employ a second specific binding partner that typically includes an antibody specific to the protein of interest. In certain embodiments, the second specific binding partner includes a detectable label conjugated to the antibody, and function as a detection conjugate.

Detectable labels suitable for use in the detection conjugate include any compound or composition having a moiety that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, or chemical means. Such labels include, for example, a radioactive label, an enzymatic label, a chemiluminescent label, a fluorescence label, a thermometric label, and an immuno-polymerase chain reaction label.

Thus for example, in an immunoassay employing an optical signal, the optical signal is measured as a protein concentration dependent change in chemiluminescence, fluorescence, phosphorescence, electrochemiluminescence, ultraviolet absorption, visible absorption, infrared absorption, refraction, surface plasmon resonance. In an immunoassay employing an electrical signal, the electrical signal is measured as an protein concentration dependent change in current, resistance, potential, mass to charge ratio, or ion count. In an immunoassay employing a change-of-state signal, the change of state signal is measured as an protein concentration dependent change in size, solubility, mass, or resonance.

More specifically, the label can be for example an enzyme, oligonucleotide, nanoparticle chemiluminophore, fluorophore, fluorescence quencher, chemiluminescence quencher, or biotin. Useful labels according to the present disclosure include magnetic beads (e.g., Dynabeads™), fluorescent dyes (e.g., fluorescein, Texas Red, rhodamine, green fluorescent protein) and the like (see, e.g., Molecular Probes, Eugene, Oreg., USA), chemiluminescent compounds such as acridinium (e.g., acridinium-9-carboxamide), phenanthridinium, dioxetanes, luminol and the like, radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), catalysts such as enzymes (e.g., horse radish peroxidase, alkaline phosphatase, beta-galactosidase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold (e.g., gold particles in the 40-80 nm diameter size range scatter green light with

high efficiency) or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

The label can be attached to the detection antibody to form the detection conjugate prior to, or during, or after contact with the biological sample. So-called "direct labels" are detectable labels that are directly attached to or incorporated into the detection antibody prior to use in the assay. Direct labels can be attached to or incorporated into the detection antibody by any of a number of means well known to those of skill in the art.

In contrast, so-called "indirect labels" typically bind to the detection antibody at some point during the assay. Often, the indirect label binds to a moiety that is attached to or incorporated into the detection agent prior to use. Thus, for example, a detection antibody can be biotinylated before use in an assay. During the assay, an avidin-conjugated fluorophore can bind the biotin-bearing detection agent, to provide a label that is easily detected.

In another example of indirect labeling, polypeptides capable of specifically binding immunoglobulin constant regions, such as polypeptide A or polypeptide G, can also be used as labels for detection antibodies. These polypeptides are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (see, generally Kronval, et al. (1973) J. Immunol., 111: 1401-1406, and Akerstrom (1985) J. Immunol., 135: 2589-2542). Such polypeptides can thus be labeled and added to the assay mixture, where they will bind to the capture and detection antibodies, as well as to the autoantibodies, labeling all and providing a composite signal attributable to protein and autoantibody present in the sample.

Some labels useful in the present disclosure may require the use of an additional reagent(s) to produce a detectable signal. In an ELISA, for example, an enzyme label (e.g., beta-galactosidase) will require the addition of a substrate (e.g., X-gal) to produce a detectable signal. In immunoassay detection methods using an acridinium compound as a direct label, a basic solution and a source of hydrogen peroxide are added.

Detection Systems - Exemplary Formats

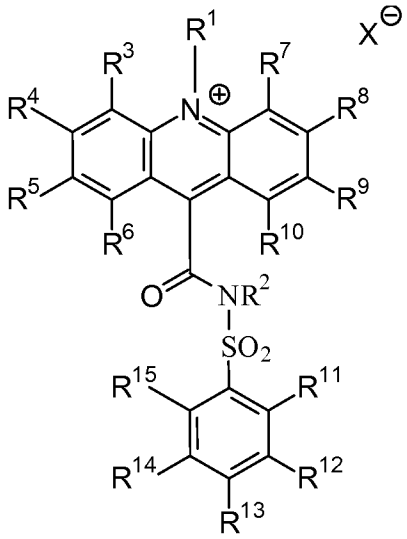
Chemiluminescence Immunoassay: In an exemplary embodiment, a chemiluminescent compound is used in the above-described methods as a direct label as part of a detection conjugate. The chemiluminescent compound can be an acridinium compound. When an acridinium compound is used as the detectable label, then the above-described

method may further include generating or providing a source of hydrogen peroxide to the mixture resulting from contacting the test sample with the first specific binding partner and the second specific binding partner (detection conjugate) and adding at least one basic solution to the mixture to generate a light signal. The light signal generated or emitted by the mixture is then measured to detect the protein of interest in the test sample.

The source of hydrogen peroxide may be a buffer solution or a solution containing hydrogen peroxide or an enzyme that generates hydrogen peroxide when added to the test sample. A hydrogen peroxide generating enzyme can be selected for example from the group consisting of: (R)-6-hydroxynicotine oxidase, (S)-2-hydroxy acid oxidase, (S)-6-hydroxynicotine oxidase, 3-aci-nitropropanoate oxidase, 3-hydroxyanthranilate oxidase, 4-hydroxymandelate oxidase, 6-hydroxynicotinate dehydrogenase, abscisic-aldehyde oxidase, acyl-CoA oxidase, alcohol oxidase, aldehyde oxidase, amine oxidase, amine oxidase (copper-containing), amine oxidase (flavin-containing), aryl-alcohol oxidase, aryl-aldehyde oxidase, catechol oxidase, cholesterol oxidase, choline oxidase, columbamine oxidase, cyclohexylamine oxidase , cytochrome c oxidase, D-amino-acid oxidase, D-arabinono-1,4-lactone oxidase, D-arabinono-1,4-lactone oxidase, D-aspartate oxidase, D-glutamate oxidase, D-glutamate(D-aspartate) oxidase, dihydrobenzophenanthridine oxidase, dihydroorotate oxidase, dihydrouracil oxidase, dimethylglycine oxidase, D-mannitol oxidase, ecdysone oxidase, ethanolamine oxidase, galactose oxidase , glucose oxidase , glutathione oxidase, glycerol-3-phosphate oxidase, glycine oxidase, glyoxylate oxidase, hexose oxidase, hydroxyphytanate oxidase, indole-3-acetaldehyde oxidase, lactic acid oxidase, L-amino-acid oxidase, L-aspartate oxidase, L-galactonolactone oxidase, L-glutamate oxidase, L-gulonolactone oxidase, L-lysine 6-oxidase, L-lysine oxidase, long-chain-alcohol oxidase, L-pipecolate oxidase, L-sorbose oxidase, malate oxidase, methanethiol oxidase, monoamino acid oxidase , N6-methyl-lysine oxidase, N-acylhexosamine oxidase, NAD(P)H oxidase, nitroalkane oxidase, N-methyl-L-amino-acid oxidase, nucleoside oxidase, oxalate oxidase, polyamine oxidase, polyphenol oxidase, polyvinyl-alcohol oxidase, prenylcysteine oxidase, protein-lysine 6-oxidase, putrescine oxidase, pyranose oxidase, pyridoxal 5′-phosphate synthase, pyridoxine 4-oxidase, pyrroloquinoline-quinone synthase, pyruvate oxidase, pyruvate oxidase (CoA-acetylating), reticuline oxidase, retinal oxidase, rifamycin-B oxidase, sarcosine oxidase, secondary-alcohol oxidase, sulfite oxidase, superoxide dismutase, superoxide reductase, tetrahydroberberine oxidase, thiamine oxidase, tryptophan α,β-oxidase, urate oxidase (uricase, uric acid oxidase), vanillyl-alcohol oxidase, xanthine oxidase, xylitol oxidase and combinations thereof.

The basic solution serves as a trigger solution, and the order in which the at least one basic solution and detectable label are added is not critical. The basic solution used in the method is a solution that contains at least one base and that has a pH greater than or equal to 10, preferably, greater than or equal to 12. Examples of basic solutions include, but are not limited to, sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium hydroxide, calcium carbonate and calcium bicarbonate. The amount of basic solution added to the test sample depends on the concentration of the basic solution used in the assay. Based on the concentration of the basic solution used, one skilled in the art could easily determine the amount of basic solution to be used in the method described herein.

In a chemiluminescence immunoassay according to the present disclosure and using an acridinium compound as the detectable label, preferably the acridinium compound is an acridinium-9-carboxamide. Specifically, the acridinium-9-carboxamide has a structure according to formula I:



I

wherein R^1 and R^2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

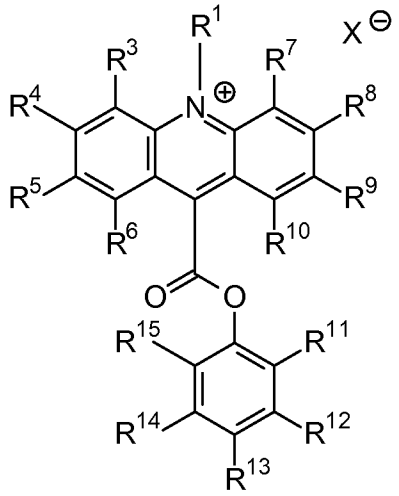
wherein R^3 through R^{15} are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl;

and further wherein any of the alkyl, alkenyl, alkynyl, aryl or aralkyl may contain one or more heteroatoms; and

optionally, if present, $X^{\ominus}$ is an anion.

Methods for preparing acridinium 9-carboxamides are described in Mattingly, P. G. J. Biolumin. Chemilumin., 6, 107-14; (1991); Adamczyk, M.; Chen, Y.-Y., Mattingly, P. G.; Pan, Y. J. Org. Chem., 63, 5636-5639 (1998); Adamczyk, M.; Chen, Y.-Y.; Mattingly, P. G.; Moore, J. A.; Shreder, K. Tetrahedron, 55, 10899-10914 (1999); Adamczyk, M.; Mattingly, P. G.; Moore, J. A.; Pan, Y. Org. Lett., 1, 779-781 (1999); Adamczyk, M.; Chen, Y.-Y.; Fishpaugh, J. R.; Mattingly, P. G.; Pan, Y.; Shreder, K.; Yu, Z. Bioconjugate Chem., 11, 714-724 (2000); Mattingly, P. G.; Adamczyk, M. In Luminescence Biotechnology: Instruments and Applications; Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77–105 (2002); Adamczyk, M.; Mattingly, P. G.; Moore, J. A.; Pan, Y. Org. Lett., 5, 3779-3782 (2003); and U.S. Patent Nos. 5,468,646, 5,543,524 and 5,783,699 (each incorporated herein by reference in their entireties for their teachings regarding same).

Alternatively, the acridinium compound can be an acridinium-9-carboxylate aryl ester; the acridinium-9-carboxylate aryl ester can have a structure according to formula II:



II

wherein R^1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R^3 through R^{15} are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion.

Examples of acridinium-9-carboxylate aryl esters having the above formula II that can be used in the present disclosure include, but are not limited to, 10-methyl-9-(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra, F., et al., Photochem. Photobiol., 4, 1111-21 (1965); Razavi, Z et al., Luminescence, 15:245-249 (2000); Razavi, Z et al., Luminescence, 15:239-244 (2000); and U.S. Patent No. 5,241,070 (each incorporated herein by reference in their entireties for their teachings regarding same).

In addition to the at least one acridinium compound, the indicator solution can also contain at least one surfactant. Any surfactant that when dissolved in water, lowers the surface tension of the water and increases the solubility of organic compounds, can be used in the present invention. Examples of surfactants that can be used is one or more non-ionic or ionic surfactants (e.g., anionic, cationic or zwitterionic surfactants). Examples of non-ionic surfactants that can be used include, but are not limited to, t-octylpheoxypolyethoxyethanol (TRITON X-100, Sigma Aldrich, St. Louis, MO), polyoxyethylenesorbitan monolaurate (Tween 20), nonylphenol polyoxyethylene ether (Nonidet P10), decyldimethylphosphine oxide (APO-10), Cyclohexyl-n-ethyl-β-D-Maltoside, Cyclohexyl-n-hexyl-β-D-Maltoside, Cyclohexyl-n-methyl-β-D-Maltoside, n-Decanoylsucrose, n-Decyl-β-D-glucopyranoside, n-Decyl-β-D-maltopyranoside, n-Decyl-β-D-thiomaltoside, Digitonin, n-Dodecanoyl sucrose, n-Dodecyl-β-D-glucopyranoside, n-Dodecyl-β-D-maltoside, polyoxyethylene (10) dodecyl ether (Genapol C-100), isotridecanol polyglycol ether (Genapol X-80), isotridecanol polyglycol ether (Genapol X-100), Heptane-1,2,3-triol, n-Heptyl-β-D-glucopyranoside, n-Heptyl-β-D-thioglucopyranoside and combinations thereof. An example of a ionic surfactant that can be used include, sodium cholate, chenodeoxycholic acid, cholic acid, dehydrocholic acid, docusate sodium, docusate sodium salt, glycocholic acid hydrate, glycodeoxycholic acid monohydrate, glycolithocholic acid ethyl ester, N-lauroylsarcosine sodium salt, N-lauroylsarcosine, lithium dodecyl sulfate, calcium propionate, 1-octanesulfonic acid sodium salt, sodium 1-butanesulfonate, sodium chenodeoxycholate, sodium cholate hydrate, sodium 1-decanesulfonate, sodium 1-decanesulfonate, sodium deoxycholate, sodium deoxycholate monohydrate, sodium dodecylbenzenesulfonate, sodium dodecyl sulfate, sodium glycochenodeoxycholate, sodium glycocholate hydrate, sodium 1-heptanesulfonate, sodium hexanesulfonate, sodium 1-nonanesulfonate, sodium octyle sulfate, sodium pentanesulfonate, sodium 1-propanesulfonate hydrate, sodium taurodeoxycholate hydrate, sodium taurohyodeoxycholate hydrate, sodium tauroursodeoxycholate, taurocholic acid sodium salt

hydrate, taurolithocholic acid 3-sulfate disodium salt, Triton® X-200, Triton® QS-15, Triton® QS-44, Triton® XQS-20, Trizma® dodecyl sulfate, ursodeoxycholic acid, alkyltrimethylammonium bromide, amprolium hydrocholoride, benzalkonium chloride, benzethonium hydroxide, benzyldimethylhexadecylammonium chloride, benzyldodecyldimethylammonium bromide, choline p-toluenesulfonate salt, dimethyldioctadecylammonium bromide, dodecylethyldimethylammonium bromide, dodecyltrimethylammonium bromide, ethylhexadecyldimethylammonium bromide, Ggirard's reagent, hexadecylpyridinium bromide, hexadecylpyridinium chloride monohydrate, hexadecylpyridinium chloride monohydrate, hexadecyltrimethylammonium bromide, hexadecyltrimethylammonium p-toluenesulfonate, hexadecyltrimethylammonium bromide, hexadecyltrimethylammonium p-toluenesulfonate, Hyamine® 1622, methylbenzethonium chloride, myristyltrimethylammonium bromide, oxyphenonium bromide, N,N',N'-polyoxyethylene (10)-N-tallow-1,3-diaminopropane, tetraheptylammonium bromide, tetrakis(decyl)ammonium bromide, thonzonium bromide and Luviquat™ FC370, Luviquat™ HM 552, Luviquat™ HOLD, Luviquat™ MS 370, Luviquat™ PQ 11PN and combinations thereof (all available from Sigma Aldrich, St. Louis, MO).

Optionally, the test sample may be treated prior to the addition of any one or more of the at least one basic solution, hydrogen peroxide source and detectable label. Such treatment may include dilution, ultrafiltration, extraction, precipitation, dialysis, chromatography and digestion. Such treatment may be in addition to and separate from any pretreatment that the test sample may receive or be subjected to as discussed previously herein. Moreover, if such treatment methods are employed with respect to the test sample, such treatment methods are such that the protein of interest remains in the test sample at a concentration proportional to that in an untreated test sample (e.g., namely, a test sample that is not subjected to any such treatment method(s)).

As mentioned briefly previously herein, the time and order in which the test sample, the at least one basic solution, source of hydrogen peroxide and the detectable label are added to form a mixture is not critical. Additionally, the mixture formed by the at least one basic solution, hydrogen peroxide source and the detectable label, can optionally be allowed to incubate for a period of time. For example, the mixture can be allowed to incubate for a period of time of from about 1 second to about 60 minutes. Specifically, the mixture can be allowed to incubate for a period of from about 1 second to about 18 minutes.

When a chemiluminescent detectable label is used, after the addition of the at least one basic solution, hydrogen peroxide source, and the detectable label to the test sample, a

detectable signal, namely, a chemiluminescent signal, is generated. The signal generated by the mixture is detected for a fixed duration of time. Preferably, the mixture is formed and the signal is detected concurrently. The duration of the detection may range from about 0.01 to about 360 seconds, more preferably from about 0.1 to about 30 seconds, and most preferably from about 0.5 to about 5 seconds. Chemiluminescent signals generated can be detected using routine techniques known to those skilled in the art.

Thus, in a chemiluminescent immunoassay according to the present disclosure, a chemiluminescent detectable label is used and added to the test sample, the chemiluminescent signal generated after the addition of the basic solution and the detectable label indicates the presence of the protein of interest in the test sample, which signal can be detected. The amount or concentration of the protein of interest in the test sample can be quantified based on the intensity of the signal generated. Specifically, the amount of the protein of interest contained in a test sample is proportional to the intensity of the signal generated. Specifically, the amount of the protein of interest present can be quantified based on comparing the amount of light generated to a standard curve for the protein of interest or by comparison to a reference standard. The standard curve can be generated using serial dilutions or solutions to the protein of interest of known concentration, by mass spectroscopy, gravimetrically and by other techniques known in the art.

Fluorescence Polarization Immunoassay (FPIA): In an exemplary embodiment, a fluorescent label is employed in a fluorescence polarization immunoassay (FPIA) according to the invention. Generally, fluorescent polarization techniques are based on the principle that a fluorescent label, when excited by plane-polarized light of a characteristic wavelength, will emit light at another characteristic wavelength (i.e., fluorescence) that retains a degree of the polarization relative to the incident light that is inversely related to the rate of rotation of the label in a given medium. As a consequence of this property, a label with constrained rotation, such as one bound to another solution component with a relatively lower rate of rotation, will retain a relatively greater degree of polarization of emitted light than when free in solution.

This technique can be employed in an immunoassay according to the present disclosure, for example, by selecting reagents such that binding of the fluorescently labeled entities forms a complex sufficiently different in size such that a change in the intensity light emitted in a given plane can be detected. For example, when a labeled cardiac troponin antibody, i.e. a second specific binding partner is bound by one or more cardiac troponin

antigens bound to the first specific binding partner, the resulting complex is sufficiently larger, and its rotation is sufficiently constrained, relative to any free labeled cardiac troponin antibody that binding is easily detected.

Fluorophores useful in FPIA include fluorescein, aminofluorescein, carboxyfluorescein, and the like, preferably 5 and 6-aminomethylfluorescein, 5 and 6-aminofluorescein, 6-carboxyfluorescein, 5-carboxyfluorescein, thioureafluorescein, and methoxytriazinolyl-aminofluorescein, and similar fluorescent derivatives. Examples of commercially available automated instruments with which fluorescence polarization assays can be conducted include: the IMx system, the TDx system, and TDxFLx system (all available from Abbott Laboratories, Abbott Park, Ill.).

Scanning Probe Microscopy (SPM): The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the immunoassay methods of the present disclosure are easily adaptable. In SPM, in particular in atomic force microscopy, the capture antibody is affixed to the solid phase that in addition to being capable of binding autoantibodies, has a surface suitable for scanning. The capture antibody can, for example, be adsorbed to a plastic or metal surface. Alternatively, the capture antibody can be covalently attached to, e.g., derivatized plastic, metal, silicon, or glass according to methods known to those of ordinary skill in the art. Following attachment of the capture antibody, the test sample is contacted with the solid phase, and a scanning probe microscope is used to detect and quantify solid phase-affixed complexes. The use of SPM eliminates the need for labels that are typically employed in immunoassay systems. Such a system is described in U.S. App. No. 662,147, which is incorporated herein by reference.

MicroElectroMechanical Systems (MEMS): Immunoassays according to the present disclosure can also be carried out using a MicroElectroMechanical System (MEMS). MEMS are microscopic structures integrated onto silicon that combine mechanical, optical, and fluidic elements with electronics, allowing convenient detection of an protein of interest. An exemplary MEMS device suitable for use in the present disclosure is the Protiveris' multicantilever array. This array is based on chemo-mechanical actuation of specially designed silicon microcantilevers and subsequent optical detection of the microcantilever deflections. When coated on one side with a binding partner, a microcantilever will bend when it is exposed to a solution containing the complementary molecule. This bending is caused by the change in the surface energy due to the binding event. Optical detection of the

degree of bending (deflection) allows measurement of the amount of complementary molecule bound to the microcantilever.

Electrochemical Detection Systems: In other embodiments, immunoassays according to the present disclosure are carried out using electrochemical detection, the techniques for which are well known to those skilled in the art. Such electrochemical detection often employs one or more electrodes connected to a device that measures and records an electrical current. Such techniques can be realized in a number of commercially available devices, such as the I-STAT® (Abbott Laboratories, Abbott Park, IL) system, which comprises a hand-held electrochemical detection instrument and self-contained assay-specific reagent cartridges. For example, in the present invention, the basic trigger solution could be contained in the self-contained hemoglobin reagent cartridge and upon addition of the test sample, a current would be generated at at least one electrode that is proportional to the amount of hemoglobin in the test sample. A basic procedure for electrochemical detection has been described for example by Heineman and coworkers. This entailed immobilization of a primary antibody (Ab, rat-anti mouse IgG), followed by exposure to a sequence of solutions containing the antigen (Ag, mouse IgG), the secondary antibody conjugated to an enzyme label (AP-Ab, rat anti mouse IgG and alkaline phosphatase), and p-aminophenyl phosphate (PAPP). The AP converts PAPP to p-aminophenol (PAP_R, the "R" is intended to distinguish the reduced form from the oxidized form, PAP_O, the quinoneimine), which is electrochemically reversible at potentials that do not interfere with reduction of oxygen and water at pH 9.0, where AP exhibits optimum activity. PAP_R does not cause electrode fouling, unlike phenol whose precursor, phenylphosphate, is often used as the enzyme substrate. Although PAP_R undergoes air and light oxidation, these are easily prevented on small scales and short time frames. Picomole detection limits for PAP_R and femtogram detection limits for IgG achieved in microelectrochemical immunoassays using PAPP volumes ranging from 20 µl to 360 µL have been reported previously. In capillary immunoassays with electrochemical detection, the lowest detection limit reported thus far is 3000 molecules of mouse IgG using a volume of 70 µL and a 30 min or 25 min assay time.

In an exemplary embodiment employing electrochemical detection according to the present disclosure, an antibody serving as the first specific binding partner, which is reactive with the protein of interest, can be immobilized on the surface of an electrode, which is the solid phase. The electrode is then contacted with a test sample from, e.g., a human. Any protein in the sample binds to the first specific binding partner, e.g. antibody to form a solid

phase-affixed complex. Autoantibodies present in the sample are blocked by the peptide reagent from interacting with the target protein and thus from interfering with binding of the target protein to the first specific binding partner. The solid phase-affixed complexes are contacted with the detection conjugate including a detectable label. Formation of an immunodetection complex that includes the first specific binding partner, protein, and detection conjugate results in generation of a signal by the detectable label, which is then detected.

Various electrochemical detection systems are described in U.S. Pat. No. 7,045,364 (issued May 16, 2006; incorporated herein by reference), U.S. Pat. No. 7,045,310 (issued May 16, 2006; incorporated herein by reference), U.S. Pat. No. 6,887,714 (issued May 3, 2005; incorporated herein by reference), U.S. Pat. No. 6,682,648 (issued Jan. 27, 2004; incorporated herein by reference); U.S. Pat. No. 6,670,115 (issued Dec. 30, 2003; incorporated herein by reference).

D. Kits

The present disclosure also provides kits for assaying test samples for presence of an protein of interest wherein the test sample may contain autoantibodies. Kits according to the present disclosure include one or more reagents useful for practicing one or more immunoassays according to the present disclosure. A kit generally includes a package with one or more containers holding the reagents, as one or more separate compositions or, optionally, as admixture where the compatibility of the reagents will allow. The test kit can also include other material(s), which may be desirable from a user standpoint, such as a buffer(s), a diluent(s), a standard(s), and/or any other material useful in sample processing, washing, or conducting any other step of the assay.

In certain embodiments, a test kit for detecting and/or quantifying at least one protein of interest in a test sample includes a capture reagent comprising an antibody that binds to the protein of interest; and instructions for detecting and/or quantifying at least one protein of interest in a test sample. The kit may further include a conjugate which includes an antibody conjugated to a detectable label.

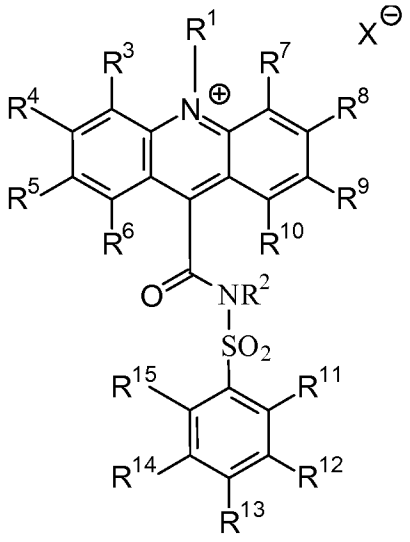
In certain embodiments, a test kit may include a humanized monoclonal antibody, wherein the humanized monoclonal antibody is specific for the protein of interest. This component can be used as a positive control in immunoassays according to the invention. If desired, this component can be included in the test kit in multiple concentrations to facilitate the generation of a standard curve to which the signal detected in the test sample can be

compared. Alternatively, a standard curve can be generated by preparing dilutions of a single humanized monoclonal antibody solution provided in the kit.

Kits according to the present disclosure can include one or more peptide reagents having a sequence derived from the protein of interest, an antibody (first specific binding partner) that binds to at least one epitope on the protein of interest, a solid phase capable of binding the first specific binding partner, a second antibody that binds to at least one epitope on the protein of interest, and instructions for detecting or quantifying the protein of interest. In certain embodiments test kits according to the present disclosure may include the solid phase as a material such as a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a quartz crystal, a film, a filter paper, a disc or a chip.

Test kits according to the present disclosure can include for example non-human monoclonal antibodies against the protein of interest, as the first and second specific binding partners. The kit may also include a detectable label that can be or is conjugated to an antibody to provide a detection conjugate as the second specific binding partner.

In certain embodiments, the test kit includes the detectable label as at least one direct label, which may be an enzyme, oligonucleotide, nanoparticle chemiluminophore, fluorophore, fluorescence quencher, chemiluminescence quencher, or biotin. In some embodiments, the direct label is an acridinium compound such as an acridinium-9-



carboxamide according to formula I:

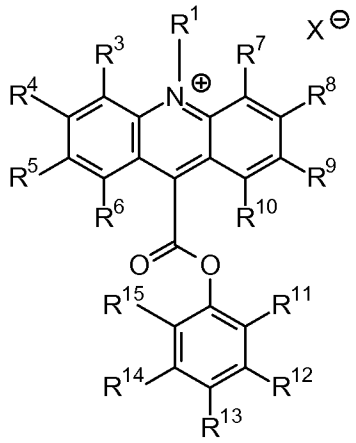
I

wherein R1 and R2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion.

Alternatively, the acridinium compound can be an acridinium-9-carboxylate aryl ester having a structure according to formula II:



II

wherein R1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion.

Test kits according to the present disclosure and which include an acridinium compound can also include a basic solution. For example, the basic solution can be a

solution having a pH of at least about 10. In certain embodiments, test kits according to the present disclosure may further include a hydrogen peroxide source, such as a buffer solution, a solution containing hydrogen peroxide, or a hydrogen peroxide generating enzyme. For example, test kits may include an amount of a hydrogen peroxide generating enzymes selected from the following: (R)-6-hydroxynicotine oxidase, (S)-2-hydroxy acid oxidase, (S)-6-hydroxynicotine oxidase, 3-aci-nitropropanoate oxidase, 3-hydroxyanthranilate oxidase, 4-hydroxymandelate oxidase, 6-hydroxynicotinate dehydrogenase, abscisic-aldehyde oxidase, acyl-CoA oxidase, alcohol oxidase, aldehyde oxidase, amine oxidase, amine oxidase (copper-containing), amine oxidase (flavin-containing), aryl-alcohol oxidase, aryl-aldehyde oxidase, catechol oxidase, cholesterol oxidase, choline oxidase, columbamine oxidase, cyclohexylamine oxidase , cytochrome c oxidase, D-amino-acid oxidase, D-arabinono-1,4-lactone oxidase, D-arabinono-1,4-lactone oxidase, D-aspartate oxidase, D-glutamate oxidase, D-glutamate(D-aspartate) oxidase, dihydrobenzophenanthridine oxidase, dihydroorotate oxidase, dihydrouracil oxidase, dimethylglycine oxidase, D-mannitol oxidase, ecdysone oxidase, ethanolamine oxidase, galactose oxidase , glucose oxidase , glutathione oxidase, glycerol-3-phosphate oxidase, glycine oxidase, glyoxylate oxidase, hexose oxidase, hydroxyphytanate oxidase, indole-3-acetaldehyde oxidase, lactic acid oxidase, L-amino-acid oxidase, L-aspartate oxidase, L-galactonolactone oxidase, L-glutamate oxidase, L-gulonolactone oxidase, L-lysine 6-oxidase, L-lysine oxidase, long-chain-alcohol oxidase, L-pipecolate oxidase, L-sorbose oxidase, malate oxidase, methanethiol oxidase, monoamino acid oxidase , N6-methyl-lysine oxidase, N-acylhexosamine oxidase, NAD(P)H oxidase, nitroalkane oxidase, N-methyl-L-amino-acid oxidase, nucleoside oxidase, oxalate oxidase, polyamine oxidase, polyphenol oxidase, polyvinyl-alcohol oxidase, prenylcysteine oxidase, protein-lysine 6-oxidase, putrescine oxidase, pyranose oxidase, pyridoxal 5′-phosphate synthase, pyridoxine 4-oxidase, pyrroloquinoline-quinone synthase, pyruvate oxidase, pyruvate oxidase (CoA-acetylating), reticuline oxidase, retinal oxidase, rifamycin-B oxidase, sarcosine oxidase, secondary-alcohol oxidase, sulfite oxidase, superoxide dismutase, superoxide reductase, tetrahydroberberine oxidase, thiamine oxidase, tryptophan α,β-oxidase, urate oxidase (uricase, uric acid oxidase), vanillyl-alcohol oxidase, xanthine oxidase, xylitol oxidase and combinations thereof.

In certain embodiments, test kits according to the present disclosure are configured for detection or quantification of one of the following specific analytes of interest cardiac troponin, thyroid stimulating hormone (TSH), beta human chorionic gonadotropin (beta-HCG); myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic

peptide (BNP), myosin light chain 2, myosin-6 and myosin-7. In such embodiments, the test kits include at least one peptide reagent having a sequence derived from the protein of interest, a first antibody and a second antibody that each bind to an epitope on the selected protein of interest, i.e. a first antibody and a second antibody and second antibody that each bind to an epitope on one of the following: cardiac troponin, thyroid stimulating hormone (TSH), beta human chorionic gonadotropin (beta-HCG); myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (BNP), myosin light chain 2, myosin-6 and myosin-7.

Test kits according to the present disclosure preferably include instructions for carrying out one or more of the immunoassays of the invention. Instructions included in kits of the present disclosure can be affixed to packaging material or can be included as a package insert. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site that provides the instructions.

E. Adaptations of the Methods of the Present Disclosure

The present disclosure is for example applicable to the jointly owned commercial Abbott Point of Care (i-STAT™) electrochemical immunoassay system which performs sandwich immunoassays for several cardiac markers, including TnI, CKMB and BNP. Immunosensors and ways of operating them in single-use test devices are described in jointly owned Publication Nos. US 20030170881, US 20040018577, US 20050054078, and US 20060160164, each of which is incorporated herein by reference. Additional background on the manufacture of electrochemical and other types of immunosensors is found in jointly owned U.S. Pat. No. 5,063,081 which is also incorporated by reference.

By way of example, and not of limitation, examples of the present disclosures shall now be given.

Example 1: Inhibition of anti-cTnI autoantibody binding to cardiac troponin-I (ELN Ref E000777-253)

Inhibitor working solutions: The peptides listed in Table 12 (obtained from Sigma-Genosys, PEPscreen custom library) were diluted in AxSYM® Troponin-I ADV

Preincubation Diluent to give solutions ranging from 240 nmol/mL to 0 nmol/mL. An equimolar mixture of the peptides listed in Table 12 was prepared and diluted to give solutions ranging from 240 nmol/mL to 0 nmol/mL/.

Table 12. Peptide inhibitors of anti-cTnI autoantibody binding to cardiac troponin-I

Peptide#	Amino-terminus	Sequence	Carboxy-terminus
1	[Btn]	SSDAAREPRPAPAPI	[NH2]
2	[Btn]	VDEERYDIEAKVTKN	[NH2]
3	[Btn]	DIEAKVTKNITEIAD	[NH2]
4	[Btn]	LDLRAHLKQVKKEDT	[NH2]
5	[Btn]	ALSGMEGRKKKFES	[NH2]

Microplate preparation: Recombinant human cardiac troponin-I (cTnI, BiosPacific, Emeryville, CA) was coated on white high-binding flat-bottom 96-well polystyrene microplates (Costar) in phosphate buffer (100 µL, 0.2 M, pH 8, 4 µg/mL) at 38 °C, for 1 h, then overcoated sequentially with bovine serum albumin and 2% wt/v sucrose in PBS.

Chemiluminescent detection conjugate: A murine anti-human IgG (subtype IgG2b, kappa;) was labeled with a chemiluminescent acridinium-9-carboxamide. This antibody recognized all human IgG subtypes while having no significant reactivity toward human IgM or IgA, or rabbit, sheep or goat IgG.

Samples: A human serum sample containing a high level of endogenous antibodies to cardiac troponin-I was mixed 1:1 with each inhibitor dilution. The solutions were arrayed in a black polypropylene microplate, sealed and stored overnight at ambient temperature.

Assay protocol: The samples, positive and low controls (10 µL) were diluted with AxSYM® Troponin-I ADV Preincubation Diluent (90 µL) and arrayed in triplicate on the microplate. After incubating at 37 °C for 2 h, the plate was washed with ARCHITECT® Wash Buffer (6×, 350 µL). The murine anti-human IgG specific monoclonal-acridinium conjugate (100 µL) was then added and the plate incubated at 37 °C for 1 h, before a final wash with ARCHITECT® Wash Buffer (6×, 350 µL).

Chemiluminescent detection: The microplate was loaded into a Mithras microplate reader (Berthold Technologies Inc, Oak Ridge, TN) equilibrated at 28 °C. The chemiluminescence signal from each well was recorded for 2 s after the sequential addition of

ARCHITECT® Pre-Trigger solution (100 µL) and ARCHITECT® Trigger solution (100 µL).

A plot of the ratio of signal to the low control (S/LC) (Figure 11) showed that peptide #5 has the greatest inhibition of the binding of endogenous antibodies to the cTnI antigen on the microplate while the mixture of peptides gave a synergistic inhibitory effect.

Example 2. Inhibition of anti-cTnI autoantibody binding to cardiac troponin-I (ELN Ref E000777-272)

The procedure of Example 1 was repeated using the peptides listed in Table 13.

Table 13. Peptide inhibitors of anti-cTnI autoantibody binding to cardiac troponin-I

Peptide#	Amino-terminus	Sequence	Carboxy-terminus
1	[Ac]	SSDAAREPRPAPAPI	[NH2]
2	[Ac]	VDEERYDIEAKVTKN	[NH2]
3	[[Ac]	DIEAKVTKNITEIAD	[NH2]
4	[Ac]	LDLRAHLKQVKKEDT	[NH2]
5	[[Ac]	ALSGMEGRKKKFES	[OH]

A plot of the ratio of signal to the low control (S/LC) (Figure 12) showed that peptide #5 has the greatest inhibition of the binding of endogenous antibodies to the cTnI antigen on the microplate in this experiment, while the mixture of peptides again gave a synergistic inhibitory effect.

One skilled in the art would readily appreciate that the peptide reagents and related methods are well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the present disclosure disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the present disclosure pertains. All patents and

publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The present disclosure illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising," "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A reagent for use in an immunoassay for determining the presence or amount of at least one protein in a test sample, the reagent comprising:

at least one peptide comprising at least 5 consecutive amino acid residues wherein the peptide is derived from said protein and further wherein said reagent is used to block the interaction between an endogenous antibody and said protein in the test sample.

2. The reagent of claim 1, wherein the protein is selected from the group consisting of: cardiac troponin I (SEQ ID NO:1), cardiac troponin T (SEQ ID NO:2), thyroid stimulating hormone (TSH) (SEQ ID NO:3), beta-human chorionic gonadotropin (beta-HCG) (SEQ ID NO:4), myeloperoxidase (MPO) (SEQ ID NO:5), prostate specific antigen (PSA) (SEQ ID NO:6), human B-type natriuretic peptide (hBNP) (SEQ ID NO:7), myosin light chain 2 (SEQ ID NO:8), myosin-6 (SEQ ID NO:9) and myosin-7 (SEQ ID NO:10).

3. The reagent of claim 1, wherein the peptide has a length of 5 consecutive amino acids to 15 consecutive amino acids.

4. The reagent of claim 1, wherein the protein is cardiac troponin I, and the peptide has a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

5. A reagent for use in an immunoassay for determining the presence or amount of at cardiac troponin I in a test sample, the reagent comprising a peptide having a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

6. A method of detecting at least one protein of interest in a test sample, the method comprising the steps of:

a. preparing a first mixture comprising a test sample suspected of containing at least one protein of interest and at least one reagent, wherein said reagent (1) is at least one peptide comprising at least 5 consecutive amino acid residues derived from said protein that binds to the antibody of interest; and (2) disrupts the interaction between an endogenous antibody in the test sample and the antigen;

b. preparing a second mixture comprising the first mixture and a first specific binding partner, wherein the first specific binding partner comprises an antibody, wherein the antibody binds with the protein of interest to form a first specific binding partner-protein complex; and

c. contacting the second mixture with a second specific binding partner, wherein the second specific binding partner comprises an antibody that has been conjugated to a detectable label and further wherein the second specific binding partner binds to the first specific binding partner-protein complex to form a first specific binding partner-protein-second specific binding partner complex; and

d. measuring the signal generated by or emitted from the detectable label and detecting the protein of interest in the test sample.

7. The method of claim 6, wherein the protein is selected from the group consisting of: cardiac troponin I, cardiac troponin T, thyroid stimulating hormone (TSH), beta-human chorionic gonadotropin (beta-HCG), myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (hBNP), myosin light chain 2, myosin-6 and myosin-7.

8. The method of claim 6, wherein the test sample is whole blood, serum or plasma.

9. The method of claim 6, wherein the first specific binding partner is immobilized to a solid phase either before or after the formation of the first specific binding partner-protein complex.

10. The method of claim 6, wherein the second specific binding partner is immobilized to a solid phase either before or after formation of the first specific binding partner-protein-second specific binding partner complex.

11. The method of claim 6, wherein the detectable label is selected from the group consisting of a radioactive label, an enzymatic label, a chemiluminescent label, a fluorescence label, a thermometric label, and an immuno-polymerase chain reaction label.

12. The method of claim 6, wherein said detectable label is an acridinium compound.

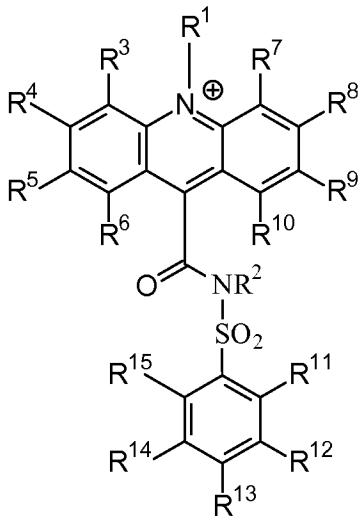
13. The method of claim 12 further comprising:

a. generating or providing a source of hydrogen peroxide to the second mixture contacted with a second specific binding partner;

b. adding a basic solution to the mixture of step (a);

c. measuring the light signal generated or emitted in step (b) and detecting the protein of interest in the sample.

14. The method of claim 12, wherein the acridinium compound is an acridinium-9-carboxamide having a structure according to formula I:



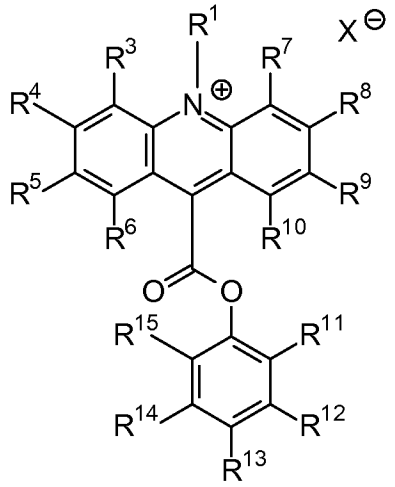
I

wherein R^1 and R^2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

wherein R^3 through R^{15} are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido,

acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and optionally, if present, X^{Θ} is an anion.

15. The method of claim 12, wherein the acridinium compound is an acridinium-9-carboxylate aryl ester having a structure according to formula II:



II

wherein R1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and optionally, if present, XΘ is an anion.

16. The method of claim 6, wherein the reagent is a peptide having a length of 5 consecutive amino acids to 15 consecutive amino acids.

17. The method of claim 6, wherein the protein is cardiac troponin I, and the peptide has a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

18. The method of claim 6, further comprising the step of quantifying the amount of protein of interest in the test sample by relating the amount of signal in step (d) to the amount of the one or more proteins of interest in the test sample either by use of a standard curve for the protein of interest or by comparison to a reference standard.

19. The method of claim 7, wherein the method is adapted for use in an automated system or semi-automated system.

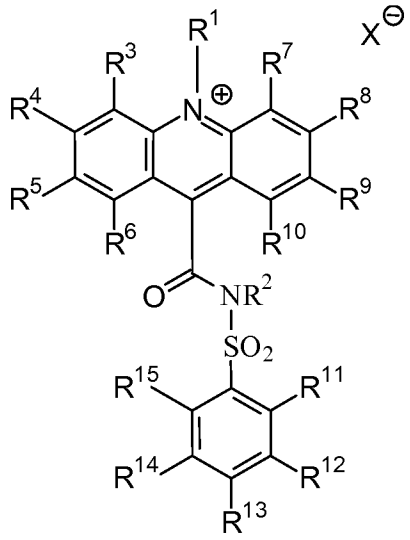
20. A kit for detecting and/or quantifying at least one protein of interest in a test sample, the kit comprising: the reagent of claim 1; a capture reagent comprising an antibody that binds to the protein of interest; and instructions for detecting and/or quantifying at least one protein of interest in a test sample.

21. The kit of claim 20, wherein the kit further comprises a conjugate comprising an antibody conjugated to a detectable label.

22. The kit of claim 21, wherein the detectable label is selected from the group consisting of a radioactive label, an enzymatic label, a chemiluminescent label, a fluorescence label, a thermometric label, and an immuno-polymerase chain reaction label.

23. The kit of claim 22, wherein the detectable label is an acridinium compound.

24. The kit of claim 23, wherein the acridinium compound is an acridinium-9-carboxamide having a structure according to formula I:



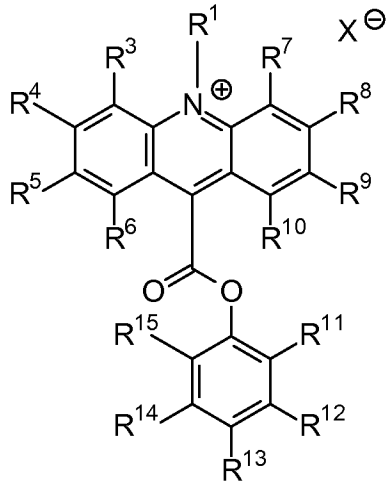
I

wherein R^1 and R^2 are each independently selected from the group consisting of: alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl, and

wherein R^3 through R^{15} are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion.

25. The kit of claim 23, wherein the acridinium compound is an acridinium-9-carboxylate aryl ester having a structure according to formula II:



II

wherein R1 is an alkyl, alkenyl, alkynyl, aryl or aralkyl, sulfoalkyl, carboxyalkyl and oxoalkyl; and

wherein R3 through R15 are each independently selected from the group consisting of: hydrogen, alkyl, alkenyl, alkynyl, aryl or aralkyl, amino, amido, acyl, alkoxyl, hydroxyl, carboxyl, halogen, halide, nitro, cyano, sulfo, sulfoalkyl, carboxyalkyl and oxoalkyl; and

optionally, if present, $X^{\ominus}$ is an anion..

26. The kit of claim 23, further comprising a basic solution.

27. The kit of claim 26, wherein the basic solution is a solution having a pH of at least about 10.

28. The kit of claim 23, further comprising a hydrogen peroxide source.

29. The kit of claim 28, wherein the hydrogen peroxide source comprises a buffer or a solution containing hydrogen peroxide.

30. The kit of claim 28, wherein the hydrogen peroxide source comprises a hydrogen peroxide generating enzyme.

31. The kit of claim 30, wherein the hydrogen peroxide generating enzyme is selected from the group consisting of: (R)-6-hydroxynicotine oxidase, (S)-2-hydroxy acid oxidase, (S)-6-hydroxynicotine oxidase, 3-aci-nitropropanoate oxidase, 3-hydroxyanthranilate oxidase, 4-hydroxymandelate oxidase, 6-hydroxynicotinate dehydrogenase, abscisic-aldehyde oxidase, acyl-CoA oxidase, alcohol oxidase, aldehyde oxidase, amine oxidase, amine oxidase (copper-containing), amine oxidase (flavin-containing), aryl-alcohol oxidase, aryl-aldehyde oxidase, catechol oxidase, cholesterol oxidase, choline oxidase, columbamine oxidase, cyclohexylamine oxidase , cytochrome c oxidase, D-amino-acid oxidase, D-arabinono-1,4-lactone oxidase, D-arabinono-1,4-lactone oxidase, D-aspartate oxidase, D-glutamate oxidase, D-glutamate(D-aspartate) oxidase, dihydrobenzophenanthridine oxidase, dihydroorotate oxidase, dihydrouracil oxidase, dimethylglycine oxidase, D-mannitol oxidase, ecdysone oxidase, ethanolamine oxidase, galactose oxidase , glucose oxidase , glutathione oxidase, glycerol-3-phosphate oxidase, glycine oxidase, glyoxylate oxidase, hexose oxidase, hydroxyphytanate oxidase, indole-3-acetaldehyde oxidase, lactic acid oxidase, L-amino-acid oxidase, L-aspartate oxidase, L-galactonolactone oxidase, L-glutamate oxidase, L-gulonolactone oxidase, L-lysine 6-oxidase, L-lysine oxidase, long-chain-alcohol oxidase, L-pipecolate oxidase, L-sorbose oxidase, malate oxidase, methanethiol oxidase, monoamino acid oxidase , N^6-methyl-lysine oxidase, N-acylhexosamine oxidase, NAD(P)H oxidase, nitroalkane oxidase, N-methyl-L-amino-acid oxidase, nucleoside oxidase, oxalate oxidase, polyamine oxidase, polyphenol oxidase, polyvinyl-alcohol oxidase, prenylcysteine oxidase, protein-lysine 6-oxidase, putrescine oxidase, pyranose oxidase, pyridoxal 5′-phosphate synthase, pyridoxine 4-oxidase, pyrroloquinoline-quinone synthase, pyruvate

oxidase, pyruvate oxidase (CoA-acetylating), reticuline oxidase, retinal oxidase, rifamycin-B oxidase, sarcosine oxidase, secondary-alcohol oxidase, sulfite oxidase, superoxide dismutase, superoxide reductase, tetrahydroberberine oxidase, thiamine oxidase, tryptophan α,β-oxidase, urate oxidase (uricase, uric acid oxidase), vanillyl-alcohol oxidase, xanthine oxidase, xylitol oxidase and combinations thereof.

32. The kit of claim 20, wherein the protein is cardiac troponin I, cardiac troponin T, thyroid stimulating hormone (TSH), beta-human chorionic gonadotropin (beta-HCG), myeloperoxidase (MPO), prostate specific antigen (PSA), human B-type natriuretic peptide (hBNP), myosin light chain 2, myosin-6 or myosin-7.

33. The kit of claim 20, wherein the reagent is a peptide having a length of 5 consecutive amino acids to 15 consecutive amino acids.

34. The kit of claim 33, wherein the protein is cardiac troponin I, and the peptide has a sequence comprising at least five consecutive amino acid residues from a sequence selected from the group consisting of SSDAAREPRPAPAPI (SEQ ID NO:11), VDEERYDIEAKVTKN (SEQ ID NO:12), DIEAKVTKNITEIAD (SEQ ID NO:13), LDLRAHLKQVKKEDT (SEQ ID NO:14), and ALSGMEGRKKKFES (SEQ ID NO:15).

Ala Asp Gly Ser Ser Asp Ala Ala Arg Glu Pro Arg Pro Ala Pro Ala Pro Ile Arg Arg Arg Ser Ser Asn Tyr Arg Ala Tyr Ala Thr Glu Pro His Ala Lys Lys Lys Ser Lys Ile Ser Ala Ser Arg Lys Leu Gln Leu Lys Thr Leu Leu Leu Gln Ile Ala Lys Gln Glu Leu Glu Arg Glu Ala Glu Glu Arg Arg Gly Glu Lys Gly Arg Ala Leu Ser Thr Arg Cys Gln Pro Leu Glu Leu Ala Gly Leu Gly Phe Ala Glu Leu Gln Asp Leu Cys Arg Gln Leu His Ala Arg Val Asp Lys Val Asp Glu Glu Arg Tyr Asp Ile Glu Ala Lys Val Thr Lys Asn Ile Thr Glu Ile Ala Asp Leu Thr Gln Lys Ile Phe Asp Leu Arg Gly Lys Phe Lys Arg Pro Thr Leu Arg Arg Val Arg Ile Ser Ala Asp Ala Met Met Gln Ala Leu Leu Gly Ala Arg Ala Lys Glu Ser Leu Asp Leu Arg Ala His Leu Lys Gln Val Lys Lys Glu Asp Thr Glu Lys Glu Asn Arg Glu Val Gly Asp Trp Arg Lys Asn Ile Asp Ala Leu Ser Gly Met Glu Gly Arg Lys Lys Lys Phe Glu

FIG. 1

Ser Asp Ile Glu Glu Val Val Glu Glu Tyr Glu Glu Glu Glu Gln Glu Glu Ala Ala Val Glu Glu Glu Glu Asp Trp Arg Glu Asp Glu Asp Glu Gln Glu Glu Ala Ala Glu Glu Asp Ala Glu Ala Glu Ala Glu Thr Glu Glu Thr Arg Ala Glu Glu Asp Glu Glu Glu Glu Glu Ala Lys Glu Ala Glu Asp Gly Pro Met Glu Glu Ser Lys Pro Lys Pro Arg Ser Phe Met Pro Asn Leu Val Pro Pro Lys Ile Pro Asp Gly Glu Arg Val Asp Phe Asp Asp Ile His Arg Lys Arg Met Glu Lys Asp Leu Asn Glu Leu Gln Ala Leu Ile Glu Ala His Phe Glu Asn Arg Lys Lys Glu Glu Glu Glu Leu Val Ser Leu Lys Asp Arg Ile Glu Arg Arg Arg Ala Glu Arg Ala Glu Gln Gln Arg Ile Arg Asn Glu Arg Glu Lys Glu Arg Gln Asn Arg Leu Ala Glu Glu Arg Ala Arg Arg Glu Glu Glu Glu Asn Arg Arg Lys Ala Glu Asp Glu Ala Arg Lys Lys Lys Ala Leu Ser Asn Met Met His Phe Gly Gly Tyr Ile Gln Lys Gln Ala Gln Thr Glu Arg Lys Ser Gly Lys Arg Gln Thr Glu Arg Glu Lys Lys Lys Lys Ile Leu Ala Glu Arg Arg Lys Val Leu Ala Ile Asp His Leu Asn Glu Asp Gln Leu Arg Glu Lys Ala Lys Glu Leu Trp Gln Ser Ile Tyr Asn Leu Glu Ala Glu Lys Phe Asp Leu Gln Glu Lys Phe Lys Gln Gln Lys Tyr Glu Ile Asn Val Leu Arg Asn Arg Ile Asn Asp Asn Gln Lys Val Ser Lys Thr Arg Gly Lys Ala Lys Val Thr Gly Arg Trp Lys

FIG. 2

Thr Ala Leu Phe Leu Met Ser Met Leu Phe Gly Leu Ala Cys Gly Gln Ala Met Ser Phe Cys Ile Pro Thr Glu Tyr Thr Met His Ile Glu Arg Arg Glu Cys Ala Tyr Cys Leu Thr Ile Asn Thr Thr Ile Cys Ala Gly Tyr Cys Met Thr Arg Asp Ile Asn Gly Lys Leu Phe Leu Pro Lys Tyr Ala Leu Ser Gln Asp Val Cys Thr Tyr Arg Asp Phe Ile Tyr Arg Thr Val Glu Ile Pro Gly Cys Pro Leu His Val Ala Pro Tyr Phe Ser Tyr Pro Val Ala Leu Ser Cys Lys Cys Gly Lys Cys Asn Thr Asp Tyr Ser Asp Cys Ile His Glu Ala Ile Lys Thr Asn Tyr Cys Thr Lys Pro Gln Lys Ser Tyr Leu Val Gly Phe Ser Val

FIG. 3

Glu Met Phe Gln Gly Leu Leu Leu Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln

FIG. 4

Gly Val Pro Phe Phe Ser Ser Leu Arg Cys Met Val Asp Leu Gly Pro Cys Trp Ala Gly Gly Leu Thr Ala Glu Met Lys Leu Leu Leu Ala Leu Ala Gly Leu Leu Ala Ile Leu Ala Thr Pro Gln Pro Ser Glu Gly Ala Ala Pro Ala Val Leu Gly Glu Val Asp Thr Ser Leu Val Leu Ser Ser Met Glu Glu Ala Lys Gln Leu Val Asp Lys Ala Tyr Lys Glu Arg Arg Glu Ser Ile Lys Gln Arg Leu Arg Ser Gly Ser Ala Ser Pro Met Glu Leu Leu Ser Tyr Phe Lys Gln Pro Val Ala Ala Thr Arg Thr Ala Val Arg Ala Ala Asp Tyr Leu His Val Ala Leu Asp Leu Leu Glu Arg Lys Leu Arg Ser Leu Trp Arg Arg Pro Phe Asn Val Thr Asp Val Leu Thr Pro Ala Gln Leu Asn Val Leu Ser Lys Ser Ser Gly Cys Ala Tyr Gln Asp Val Gly Val Thr Cys Pro Glu Gln Asp Lys Tyr Arg Thr Ile Thr Gly Met Cys Asn Asn Arg Arg Ser Pro Thr Leu Gly Ala Ser Asn Arg Ala Phe Val Arg Trp Leu Pro Ala Glu Tyr Glu Asp Gly Phe Ser Leu Pro Tyr Gly Trp Thr Pro Gly Val Lys Arg Asn Gly Phe Pro Val Ala Leu Ala Arg Ala Val Ser Asn Glu Ile Val Arg Phe Pro Thr Asp Gln Leu Thr Pro Asp Gln Glu Arg Ser Leu Met Phe Met Gln Trp Gly Gln Leu Leu Asp His Asp Leu Asp Phe Thr Pro Glu Pro Ala Ala Arg Ala Ser Phe Val Thr Gly Val Asn Cys Glu Thr Ser Cys Val Gln Gln Pro Pro Cys Phe Pro Leu Lys Ile Pro Pro Asn Asp Pro Arg Ile Lys Asn Gln Ala Asp Cys Ile Pro Phe Phe Arg Ser Cys Pro Ala Cys Pro Gly Ser Asn Ile Thr Ile Arg Asn Gln Ile Asn Ala Leu Thr Ser Phe Val Asp Ala Ser Met Val Tyr Gly Ser Glu Glu Pro Leu Ala Arg Asn Leu Arg Asn Met Ser Asn Gln Leu Gly Leu Leu Ala Val Asn Gln Arg Phe Gln Asp Asn Gly Arg Ala Leu Leu Pro Phe Asp Asn Leu His Asp Asp Pro Cys Leu Leu Thr Asn Arg Ser Ala Arg Ile Pro Cys Phe Leu Ala Gly Asp Thr Arg Ser Ser Glu Met Pro Glu Leu Thr Ser Met His Thr Leu Leu Leu Arg Glu His Asn Arg Leu Ala Thr Glu Leu Lys Ser Leu Asn Pro Arg Trp Asp Gly Glu Arg Leu Tyr Gln Glu Ala Arg Lys Ile Val Gly Ala Met Val Gln Ile Ile Thr Tyr Arg Asp Tyr Leu Pro Leu Val Leu Gly Pro Thr Ala Met Arg Lys Tyr Leu Pro Thr Tyr Arg Ser Tyr Asn Asp Ser Val Asp Pro Arg Ile Ala Asn Val Phe Thr Asn Ala Phe Arg Tyr Gly His Thr Leu Ile Gln Pro Phe Met Phe Arg Leu Asp Asn Arg Tyr Gln Pro Met Glu Pro Asn Pro Arg Val Pro Leu Ser Arg Val Phe Phe Ala Ser Trp Arg Val Val Leu Glu Gly Gly Ile Asp Pro Ile Leu Arg Gly Leu Met Ala Thr Pro Ala Lys Leu Asn Arg Gln Asn Gln Ile Ala Val Asp Glu Ile Arg Glu Arg Leu Phe Glu Gln Val Met Arg Ile Gly Leu Asp Leu Pro Ala Leu Asn Met Gln Arg Ser Arg Asp His Gly Leu Pro Gly Tyr Asn Ala Trp Arg Arg Phe Cys Gly Leu Pro Gln Pro Glu Thr Val Gly Gln Leu Gly Thr Val Leu Arg Asn Leu Lys Leu Ala Arg Lys Leu Met Glu Gln Tyr Gly Thr Pro Asn Asn Ile Asp Ile Trp Met Gly Gly Val Ser Glu Pro Leu Lys Arg Lys Gly Arg Val Gly Pro Leu Leu Ala Cys Ile Ile Gly Thr Gln Phe Arg Lys Leu Arg Asp Gly Asp Arg Phe Trp Trp Glu Asn Glu Gly Val Phe Ser Met Gln Gln Arg Gln Ala Leu Ala Gln Ile Ser Leu Pro Arg Ile Ile Cys Asp Asn Thr Gly Ile Thr Thr Val Ser Lys Asn Asn Ile Phe Met Ser Asn Ser Tyr Pro Arg Asp Phe Val Asn Cys Ser Thr Leu Pro Ala Leu Asn Leu Ala Ser Trp Arg Glu Ala Ser

FIG. 5

Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro

FIG. 6

Asp Pro Gln Thr Ala Pro Ser Arg Ala Leu Leu Leu Leu Leu Phe Leu His Leu Ala Phe Leu Gly Gly Arg Ser His Pro Leu Gly Ser Pro Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Leu Gln Glu Gln Arg Asn His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His

FIG. 7

Ala Pro Lys Lys Ala Lys Lys Arg Ala Gly Gly Ala Asn Ser Asn Val Phe Ser Met Phe Glu Gln Thr Gln Ile Gln Glu Phe Lys Glu Ala Phe Thr Ile Met Asp Gln Asn Arg Asp Gly Phe Ile Asp Lys Asn Asp Leu Arg Asp Thr Phe Ala Ala Leu Gly Arg Val Asn Val Lys Asn Glu Glu Ile Asp Glu Met Ile Lys Glu Ala Pro Gly Pro Ile Asn Phe Thr Val Phe Leu Thr Met Phe Gly Glu Lys Leu Lys Gly Ala Asp Pro Glu Glu Thr Ile Leu Asn Ala Phe Lys Val Phe Asp Pro Glu Gly Lys Gly Val Leu Lys Ala Asp Tyr Val Arg Glu Met Leu Thr Thr Gln Ala Glu Arg Phe Ser Lys Glu Glu Val Asp Gln Met Phe Ala Ala Phe Pro Pro Asp Val Thr Gly Asn Leu Asp Tyr Lys Asn Leu Val His Ile Ile Thr His Gly Glu Glu Lys Asp

FIG. 8

Thr Asp Ala Gln Met Ala Asp Phe Gly Ala Ala Ala Gln Tyr Leu Arg Lys Ser Glu Lys Glu Arg Leu Glu Ala Gln Thr Arg Pro Phe Asp Ile Arg Thr Glu Cys Phe Val Pro Asp Asp Lys Glu Glu Phe Val Lys Ala Lys Ile Leu Ser Arg Glu Gly Gly Lys Val Ile Ala Glu Thr Glu Asn Gly Lys Thr Val Thr Val Lys Glu Asp Gln Val Leu Gln Gln Asn Pro Pro Lys Phe Asp Lys Ile Gln Asp Met Ala Met Leu Thr Phe Leu His Glu Pro Ala Val Leu Phe Asn Leu Lys Glu Arg Tyr Ala Ala Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro Tyr Lys Trp Leu Pro Val Tyr Asn Ala Glu Val Val Ala Ala Tyr Arg Gly Lys Lys Arg Ser Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp Asn Ala Tyr Gln Tyr Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val Ile Gln Tyr Phe Ala Ser Ile Ala Ala Ile Gly Asp Arg Gly Lys Lys Asp Asn Ala Asn Ala Asn Lys Gly Thr Leu Glu Asp Gln Ile Ile Gln Ala Asn Pro Ala Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly Ala Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu Lys Ser Arg Val Ile Phe Gln Leu Lys Ala Glu Arg Asn Tyr His Ile Phe Tyr Gln Ile Leu Ser Asn Lys Lys Pro Glu Leu Leu Asp Met Leu Leu Val Thr Asn Asn Pro Tyr Asp Tyr Ala Phe Val Ser Gln Gly Glu Val Ser Val Ala Ser Ile Asp Asp Ser Glu Glu Leu Met Ala Thr Asp Ser Ala Phe Asp Val Leu Gly Phe Thr Ser Glu Glu Lys Ala Gly Val Tyr Lys Leu Thr Gly Ala Ile Met His Tyr Gly Asn Met Lys Phe Lys Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Asp Ala Asp Lys Ser Ala Tyr Leu Met Gly Leu Asn Ser Ala Asp Leu Leu Lys Gly Leu Cys His Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys Gly Gln Ser Val Gln Gln Val Tyr Tyr Ser Ile Gly Ala Leu Ala Lys Ala Val Tyr Glu Lys Met Phe Asn Trp Met Val Thr Arg Ile Asn Ala Thr Leu Glu Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Phe Glu Gln Leu Cys Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr Phe Ile Asp Phe Gly Met Asp Leu Gln Ala Cys Ile Asp Leu Ile Glu Lys Pro Met Gly Ile Met Ser Ile Leu Glu Glu Glu Cys Met Phe Pro Lys Ala Thr Asp Met Thr Phe Lys Ala Lys Leu Tyr Asp Asn His Leu Gly Lys Ser Asn Asn Phe Gln Lys Pro Arg Asn Ile Lys Gly Lys Gln Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Thr Val Asp Tyr Asn Ile Leu Gly Trp Leu Glu Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val Ala Leu Tyr Gln Lys Ser Ser Leu Lys Leu Met Ala Thr Leu Phe Ser Ser Tyr Ala Thr Ala Asp Thr Gly Asp Ser Gly Lys Ser Lys Gly Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu His Arg Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Thr Thr His Pro His Phe Val Arg Cys Ile Ile Pro Asn Glu Arg Lys Ala Pro Gly Val Met Asp Asn Pro Leu Val Met His Gln Leu Arg Cys Asn Gly Val Leu Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Asn Arg Ile Leu Tyr Gly Asp Phe Arg Gln Arg Tyr Arg Ile Leu Asn Pro Val Ala Ile Pro Glu Gly Gln Phe Ile Asp Ser Arg Lys Gly Thr Glu Lys Leu Leu Ser Ser Leu Asp Ile Asp His Asn Gln Tyr Lys Phe Gly His Thr Lys Val Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Glu Arg Leu Ser Arg Ile Ile Thr Arg Met Gln Ala Gln Ala Arg Gly Gln Leu Met Arg Ile Glu Phe Lys Lys Ile Val Glu Arg Arg Asp Ala Leu Leu Val Ile Gln Trp Asn Ile Arg Ala Phe Met Gly

FIG. 9A

Val Lys Asn Trp Pro Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu Thr Glu Lys Glu Met Ala Thr Met Lys Glu Glu Phe Gly Arg Ile Lys Glu Thr Leu Glu Lys Ser Glu Ala Arg Arg Lys Glu Leu Glu Glu Lys Met Val Ser Leu Leu Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln Ala Glu Gln Asp Asn Leu Asn Asp Ala Glu Glu Arg Cys Asp Gln Leu Ile Lys Asn Lys Ile Gln Leu Glu Ala Lys Val Lys Glu Met Asn Glu Arg Leu Glu Asp Glu Glu Glu Met Asn Ala Glu Leu Thr Ala Lys Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Ile Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln Gln Ala Leu Asp Asp Leu Gln Val Glu Glu Asp Lys Val Asn Ser Leu Ser Lys Ser Lys Val Lys Leu Glu Gln Gln Val Asp Asp Leu Glu Gly Ser Leu Glu Gln Glu Lys Lys Val Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Thr Gln Glu Ser Ile Met Asp Leu Glu Asn Asp Lys Leu Gln Leu Glu Glu Lys Leu Lys Lys Lys Glu Phe Asp Ile Asn Gln Gln Asn Ser Lys Ile Glu Asp Glu Gln Ala Leu Ala Leu Gln Leu Gln Lys Lys Leu Lys Glu Asn Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Leu Glu Ala Glu Arg Thr Ala Arg Ala Lys Val Glu Lys Leu Arg Ser Asp Leu Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly Gly Ala Thr Ser Val Gln Ile Glu Met Asn Lys Lys Arg Glu Ala Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr Leu Gln His Glu Ala Thr Ala Ala Ala Leu Arg Lys Lys His Ala Asp Ser Val Ala Glu Leu Gly Glu Gln Ile Asp Asn Leu Gln Arg Val Lys Gln Lys Leu Glu Lys Glu Lys Ser Glu Phe Lys Leu Glu Leu Asp Asp Val Thr Ser Asn Met Glu Gln Ile Ile Lys Ala Lys Ala Asn Leu Glu Lys Val Ser Arg Thr Leu Glu Asp Gln Ala Asn Glu Tyr Arg Val Lys Leu Glu Glu Ala Gln Arg Ser Leu Asn Asp Phe Thr Thr Gln Arg Ala Lys Leu Gln Thr Glu Asn Gly Glu Leu Ala Arg Gln Leu Glu Glu Lys Glu Ala Leu Ile Ser Gln Leu Thr Arg Gly Lys Leu Ser Tyr Thr Gln Gln Met Glu Asp Leu Lys Arg Gln Leu Glu Glu Glu Gly Lys Ala Lys Asn Ala Leu Ala His Ala Leu Gln Ser Ala Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu Glu Glu Thr Glu Ala Lys Ala Glu Leu Gln Arg Val Leu Ser Lys Ala Asn Ser Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp Ala Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu Ala Gln Arg Leu Gln Asp Ala Glu Glu Ala Val Glu Ala Val Asn Ala Lys Cys Ser Ser Leu Glu Lys Thr Lys His Arg Leu Gln Asn Glu Ile Glu Asp Leu Met Val Asp Val Glu Arg Ser Asn Ala Ala Ala Ala Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Ile Leu Ala Glu Trp Lys Gln Lys Tyr Glu Glu Ser Gln Ser Glu Leu Glu Ser Ser Gln Lys Glu Ala Arg Ser Leu Ser Thr Glu Leu Phe Lys Leu Lys Asn Ala Tyr Glu Glu Ser Leu Glu His Leu Glu Thr Phe Lys Arg Glu Asn Lys Asn Leu Gln Glu Glu Ile Ser Asp Leu Thr Glu Gln Leu Gly Glu Gly Gly Lys Asn Val His Glu Leu Glu Lys Val Arg Lys Gln Leu Glu Val Glu Lys Leu Glu Leu Gln Ser Ala Leu Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile Leu Arg Ala Gln Leu Glu Phe Asn Gln Ile Lys Ala Glu Ile Glu Arg Lys Leu Ala Glu Lys Asp Glu Glu Met Glu Gln Ala Lys Arg Asn His Gln Arg Val Val Asp Ser Leu Gln Thr Ser Leu Asp Ala Glu Thr Arg Ser Arg Asn Glu Val Leu Arg Val Lys Lys Lys Met Glu Gly

FIG. 9B

Asp Leu Asn Glu Met Glu Ile Gln Leu Ser His Ala Asn Arg Met Ala Ala Glu Ala Gln Lys Gln Val Lys Ser Leu Gln Ser Leu Leu Lys Asp Thr Gln Ile Gln Leu Asp Asp Ala Val Arg Ala Asn Asp Asp Leu Lys Glu Asn Ile Ala Ile Val Glu Arg Arg Asn Asn Leu Leu Gln Ala Glu Leu Glu Glu Leu Arg Ala Val Val Glu Gln Thr Glu Arg Ser Arg Lys Leu Ala Glu Gln Glu Leu Ile Glu Thr Ser Glu Arg Val Gln Leu Leu His Ser Gln Asn Thr Ser Leu Ile Asn Gln Lys Lys Lys Met Glu Ala Asp Leu Thr Gln Leu Gln Ser Glu Val Glu Glu Ala Val Gln Glu Cys Arg Asn Ala Glu Glu Lys Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys Asn Met Glu Gln Thr Ile Lys Asp Leu Gln His Arg Leu Asp Glu Ala Glu Gln Ile Ala Leu Lys Gly Gly Lys Lys Gln Leu Gln Lys Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Leu Glu Ala Glu Gln Lys Arg Asn Ala Glu Ser Val Lys Gly Met Arg Lys Ser Glu Arg Arg Ile Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Lys Lys Asn Leu Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Leu Lys Val Lys Ala Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ala Asn Thr Asn Leu Ser Lys Phe Arg Lys Val Gln His Glu Leu Asp Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys Leu Arg Ala Lys Ser Arg Asp Ile Gly Ala Lys Gln Lys Met His Asp Glu Glu

FIG. 9C

Gly Asp Ser Glu Met Ala Val Phe Gly Ala Ala Ala Pro Tyr Leu Arg Lys Ser Glu Lys
Glu Arg Leu Glu Ala Gln Thr Arg Pro Phe Asp Leu Lys Lys Asp Val Phe Val Pro Asp
Asp Lys Gln Glu Phe Val Lys Ala Lys Ile Val Ser Arg Glu Gly Gly Lys Val Thr Ala Glu
Thr Glu Tyr Gly Lys Thr Val Thr Val Lys Glu Asp Gln Val Met Gln Gln Asn Pro Pro
Lys Phe Asp Lys Ile Glu Asp Met Ala Met Leu Thr Phe Leu His Glu Pro Ala Val Leu
Tyr Asn Leu Lys Asp Arg Tyr Gly Ser Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val
Thr Val Asn Pro Tyr Lys Trp Leu Pro Val Tyr Thr Pro Glu Val Val Ala Ala Tyr Arg Gly
Lys Lys Arg Ser Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp Asn Ala Tyr Gln Tyr Met
Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val
Asn Thr Lys Arg Val Ile Gln Tyr Phe Ala Val Ile Ala Ala Ile Gly Asp Arg Ser Lys Lys
Asp Gln Ser Pro Gly Lys Gly Thr Leu Glu Asp Gln Ile Ile Gln Ala Asn Pro Ala Leu Glu
Ala Phe Gly Asn Ala Lys Thr Val Arg Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile
Arg Ile His Phe Gly Ala Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
Lys Ser Arg Val Ile Phe Gln Leu Lys Ala Glu Arg Asp Tyr His Ile Phe Tyr Gln Ile
Leu Ser Asn Lys Lys Pro Glu Leu Leu Asp Met Leu Leu Ile Thr Asn Asn Pro Tyr Asp
Tyr Ala Phe Ile Ser Gln Gly Glu Thr Thr Val Ala Ser Ile Asp Asp Ala Glu Glu Leu Met
Ala Thr Asp Asn Ala Phe Asp Val Leu Gly Phe Thr Ser Glu Glu Lys Asn Ser Met Tyr
Lys Leu Thr Gly Ala Ile Met His Phe Gly Asn Met Lys Phe Lys Leu Lys Gln Arg
Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Glu Ala Asp Lys Ser Ala Tyr Leu Met Gly
Leu Asn Ser Ala Asp Leu Leu Lys Gly Leu Cys His Pro Arg Val Lys Val Gly Asn Glu
Tyr Val Thr Lys Gly Gln Asn Val Gln Gln Val Ile Tyr Ala Thr Gly Ala Leu Ala Lys Ala
Val Tyr Glu Arg Met Phe Asn Trp Met Val Thr Arg Ile Asn Ala Thr Leu Glu Thr
Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp Ile Ala Gly Phe Glu Ile Phe Asp Phe
Asn Ser Phe Glu Gln Leu Cys Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn
His His Met Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr Phe Ile
Asp Phe Gly Met Asp Leu Gln Ala Cys Ile Asp Leu Ile Glu Lys Pro Met Gly Ile Met Ser
Ile Leu Glu Glu Glu Cys Met Phe Pro Lys Ala Thr Asp Met Thr Phe Lys Ala Lys Leu
Phe Asp Asn His Leu Gly Lys Ser Ala Asn Phe Gln Lys Pro Arg Asn Ile Lys Gly Lys
Pro Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Ile Val Asp Tyr Asn Ile Ile Gly Trp
Leu Gln Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val Gly Leu Tyr Gln Lys Ser Ser
Leu Lys Leu Leu Ser Thr Leu Phe Ala Asn Tyr Ala Gly Ala Asp Ala Pro Ile Glu Lys Gly
Lys Gly Lys Ala Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu His Arg Glu Asn Leu
Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His Phe Val Arg Cys Ile Ile Pro Asn
Glu Thr Lys Ser Pro Gly Val Met Asp Asn Pro Leu Val Met His Gln Leu Arg Cys Asn
Gly Val Leu Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Asn Arg Ile Leu Tyr Gly Asp
Phe Arg Gln Arg Tyr Arg Ile Leu Asn Pro Ala Ala Ile Pro Glu Gly Gln Phe Ile Asp
Ser Arg Lys Gly Ala Glu Lys Leu Leu Ser Ser Leu Asp Ile Asp His Asn Gln Tyr Lys Phe
Gly His Thr Lys Val Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp
Glu Arg Leu Ser Arg Ile Ile Thr Arg Ile Gln Ala Gln Ser Arg Gly Val Leu Ala Arg Met

FIG. 10A

Glu Tyr Lys Lys Leu Leu Glu Arg Arg Asp Ser Leu Leu Val Ile Gln Trp Asn Ile Arg Ala Phe Met Gly Val Lys Asn Trp Pro Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu Arg Glu Lys Glu Met Ala Ser Met Lys Glu Glu Phe Thr Arg Leu Lys Glu Ala Leu Glu Lys Ser Glu Ala Arg Arg Lys Glu Leu Glu Glu Lys Met Val Ser Leu Leu Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln Ala Glu Gln Asp Asn Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu Ile Lys Asn Lys Ile Gln Leu Glu Ala Lys Val Lys Glu Met Asn Glu Arg Leu Glu Asp Glu Glu Glu Met Asn Ala Glu Leu Thr Ala Lys Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Arg Asp Ile Asp Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Ile Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln Gln Ala Leu Asp Asp Leu Gln Ala Glu Glu Asp Lys Val Asn Thr Leu Thr Lys Ala Lys Val Lys Leu Glu Gln Gln Val Asp Asp Leu Glu Gly Ser Leu Glu Gln Glu Lys Lys Val Arg Met Asp Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Thr Gln Glu Ser Ile Met Asp Leu Glu Asn Asp Lys Gln Gln Leu Asp Glu Arg Leu Lys Lys Lys Asp Phe Glu Leu Asn Ala Leu Asn Ala Arg Ile Glu Asp Glu Gln Ala Leu Gly Ser Gln Leu Gln Lys Lys Leu Lys Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Leu Glu Ala Glu Arg Thr Ala Arg Ala Lys Val Glu Lys Leu Arg Ser Asp Leu Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly Gly Ala Thr Ser Val Gln Ile Glu Met Asn Lys Lys Arg Glu Ala Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr Leu Gln His Glu Ala Thr Ala Ala Ala Leu Arg Lys Lys His Ala Asp Ser Val Ala Glu Leu Gly Glu Gln Ile Asp Asn Leu Gln Arg Val Lys Gln Lys Leu Glu Lys Glu Lys Ser Glu Phe Lys Leu Glu Leu Asp Asp Val Thr Ser Asn Met Glu Gln Ile Ile Lys Ala Lys Ala Asn Leu Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Met Asn Glu His Arg Ser Lys Ala Glu Glu Thr Gln Arg Ser Val Asn Asp Leu Thr Ser Gln Arg Ala Lys Leu Gln Thr Glu Asn Gly Glu Leu Ser Arg Gln Leu Asp Glu Lys Glu Ala Leu Ile Ser Gln Leu Thr Arg Gly Lys Leu Thr Tyr Thr Gln Gln Leu Glu Asp Leu Lys Arg Gln Leu Glu Glu Glu Val Lys Ala Lys Asn Ala Leu Ala His Ala Leu Gln Ser Ala Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu Glu Glu Thr Glu Ala Lys Ala Glu Leu Gln Arg Val Leu Ser Lys Ala Asn Ser Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp Ala Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu Ala Gln Arg Leu Gln Glu Ala Glu Glu Ala Val Glu Ala Val Asn Ala Lys Cys Ser Ser Leu Glu Lys Thr Lys His Arg Leu Gln Asn Glu Ile Glu Asp Leu Met Val Asp Val Glu Arg Ser Asn Ala Ala Ala Ala Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Ile Leu Ala Glu Trp Lys Gln Lys Tyr Glu Glu Ser Gln Ser Glu Leu Glu Ser Ser Gln Lys Glu Ala Arg Ser Leu Ser Thr Glu Leu Phe Lys Leu Lys Asn Ala Tyr Glu Glu Ser Leu Glu His Leu Glu Thr Phe Lys Arg Glu Asn Lys Asn Leu Gln Glu Glu Ile Ser Asp Leu Thr Glu Gln Leu Gly Ser Ser Gly Lys Thr Ile His Glu Leu Glu Lys Val Arg Lys Gln Leu Glu Ala Glu Lys Met Glu Leu Gln Ser Ala Leu Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys

FIG. 10B

Ile Leu Arg Ala Gln Leu Glu Phe Asn Gln Ile Lys Ala Glu Ile Glu Arg Lys Leu Ala Glu Lys Asp Glu Glu Met Glu Gln Ala Lys Arg Asn His Leu Arg Val Val Asp Ser Leu Gln Thr Ser Leu Asp Ala Glu Thr Arg Ser Arg Asn Glu Ala Leu Arg Val Lys Lys Lys Met Glu Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Ser His Ala Asn Arg Met Ala Ala Glu Ala Gln Lys Gln Val Lys Ser Leu Gln Ser Leu Leu Lys Asp Thr Gln Ile Gln Leu Asp Asp Ala Val Arg Ala Asn Asp Asp Leu Lys Glu Asn Ile Ala Ile Val Glu Arg Arg Asn Asn Leu Leu Gln Ala Glu Leu Glu Glu Leu Arg Ala Val Val Glu Gln Thr Glu Arg Ser Arg Lys Leu Ala Glu Gln Glu Leu Ile Glu Thr Ser Glu Arg Val Gln Leu Leu His Ser Gln Asn Thr Ser Leu Ile Asn Gln Lys Lys Lys Met Asp Ala Asp Leu Ser Gln Leu Gln Thr Glu Val Glu Glu Ala Val Gln Glu Cys Arg Asn Ala Glu Glu Lys Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys Asn Met Glu Gln Thr Ile Lys Asp Leu Gln His Arg Leu Asp Glu Ala Glu Gln Ile Ala Leu Lys Gly Gly Lys Lys Gln Leu Gln Lys Leu Glu Ala Arg Val Arg Glu Leu Glu Asn Glu Leu Glu Ala Glu Gln Lys Arg Asn Ala Glu Ser Val Lys Gly Met Arg Lys Ser Glu Arg Arg Ile Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys Asn Leu Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Leu Lys Val Lys Ala Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ala Asn Thr Asn Leu Ser Lys Phe Arg Lys Val Gln His Glu Leu Asp Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys Leu Arg Ala Lys Ser Arg Asp Ile Gly Thr Lys Gly Leu Asn Glu Glu

FIG. 10C

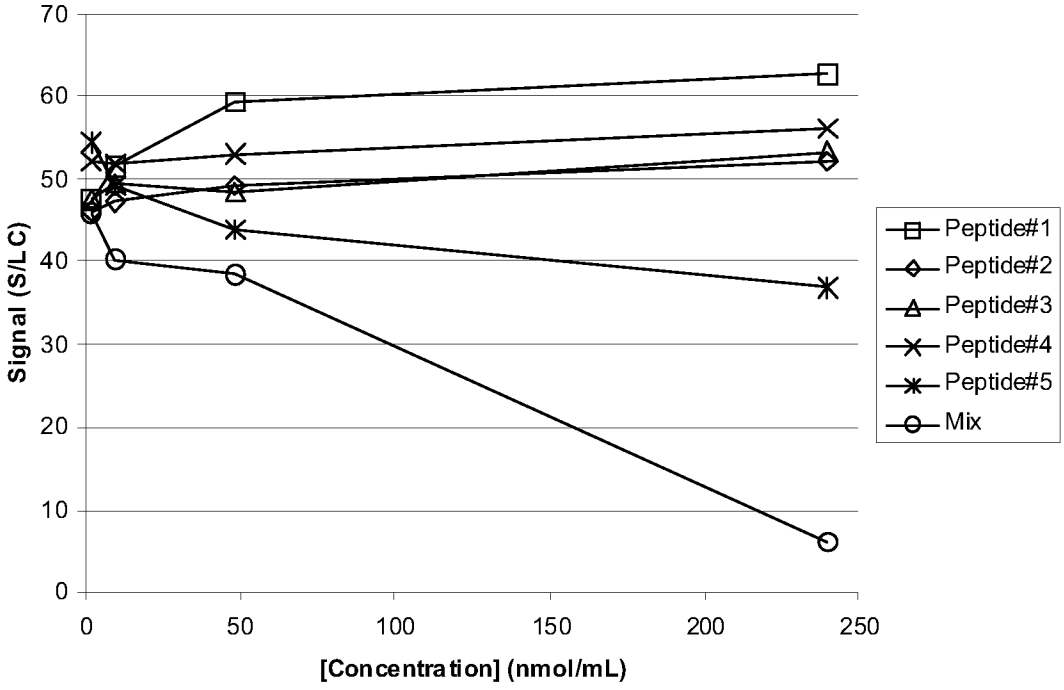

70
60
50
40
30
20
10
0
Signal (S/LC)
0
50
100
150
200
250
[Concentration] (nmol/mL)
Peptide#1
Peptide#2
Peptide#3
Peptide#4
Peptide#5
Mix

FIG. 11

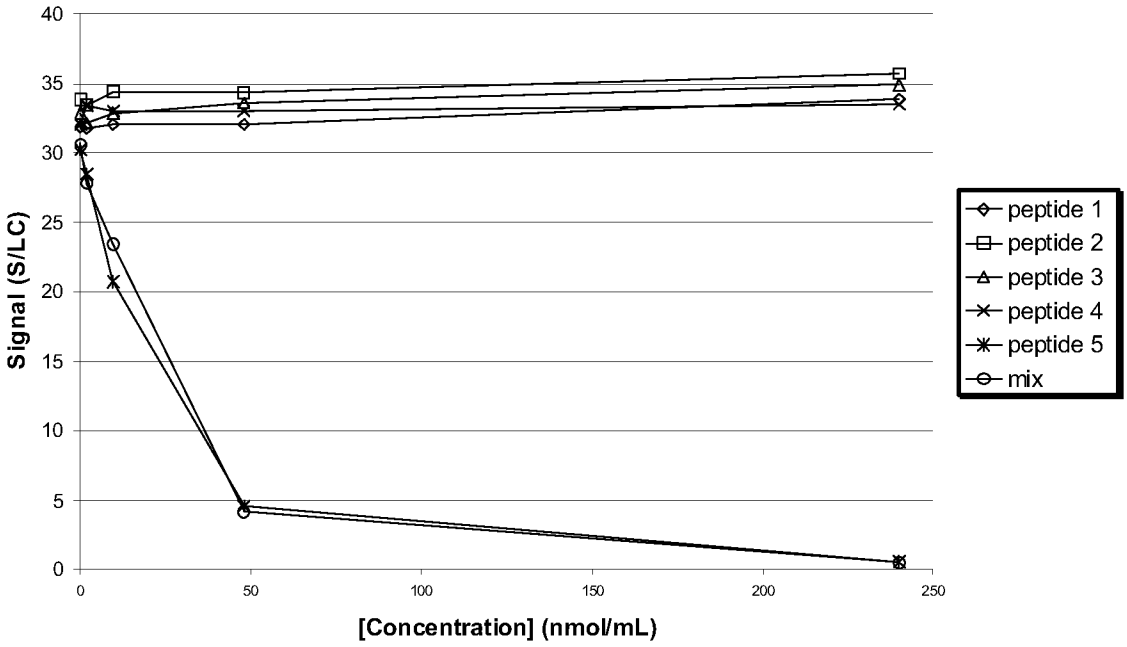

40
35
30
25
20
15
10
5
0
Signal (S/LC)
0
50
100
150
200
250
[Concentration] (nmol/mL)
peptide 1
peptide 2
peptide 3
peptide 4
peptide 5
mix

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/056943

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/53
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATWYLER SAUL A ET AL: "Potential interference by antineutrophil cytoplasmic autoantibodies in myeloperoxidase immunoassays", CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY, WASHINGTON, DC, vol. 54, no. 12, 1 December 2008 (2008-12-01), pages 2084-2086, XP009114933, ISSN: 0009-9147, DOI: DOI:10.1373/CLINCHEM.2008.110841 the whole document	1-34
A	WO 2008/051762 A2 (ABBOTT LAB [US]; MATTINGLY PHILLIP G [US]; ADAMCZYK MACIEJ [US]; BRASH) 2 May 2008 (2008-05-02) the whole document	1-34

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 2010	03/01/2011

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/056943

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/051761 A2 (ABBOTT LAB [US]; MATTINGLY PHILLIP G [US]; ADAMCZYK MACIEJ [US]; BRASH) 2 May 2008 (2008-05-02)	1-5, 20-23, 32-34
Y	the whole document	31
X	US 2009/162876 A1 (ADAMCZYK MACIEJ [US] ET AL) 25 June 2009 (2009-06-25)	1-3, 20-30, 32,33
Y	the whole document	31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/056943

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008051762 A2	02-05-2008	CA 2667012 A1	02-05-2008
		EP 2097750 A2	09-09-2009
		JP 2010508516 T	18-03-2010
		US 2009246800 A1	01-10-2009
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		JP 2010508515 T	18-03-2010
		US 2009017560 A1	15-01-2009
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		US 2010311079 A1	09-12-2010
US 2009162876 A1	25-06-2009	EP 2235204 A1	06-10-2010
		WO 2009085883 A1	09-07-2009

专利名称(译)	肽试剂和抑制自身抗体抗原结合的方法		
公开(公告)号	EP2507626A1	公开(公告)日	2012-10-10
申请号	EP2010779443	申请日	2010-11-17
[标]申请(专利权)人(译)	雅培公司		
申请(专利权)人(译)	亚培		
当前申请(专利权)人(译)	亚培		
[标]发明人	ADAMCZYK MACIEJ BRASHEAR ROY JEFFREY MATTINGLY PHILLIP G		
发明人	ADAMCZYK, MACIEJ BRASHEAR, ROY JEFFREY MATTINGLY, PHILLIP G.		
IPC分类号	G01N33/53		
CPC分类号	G01N33/5306		
优先权	12/630671 2009-12-03 US		
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