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PEPTIDES EFFECTIVE FOR DIAGNOSIS
AND DETECTION OF HEPATITIS C INFECTION

FIELD OF THE INVENTION

The present invention relates to novel linear and branched peptides specific for the diagnosis and prevention of hepatitis C virus (HCV) infection. More particularly, the present invention is directed to linear and branched synthetic peptides containing at least one antigenic site which is effective for detecting HCV-associated antibodies in patients using immunoassay techniques. In some cases, these peptides are also library peptides with degenerate amino acid positions.

BACKGROUND OF THE INVENTION

Non-A, non-B hepatitis (NANBH) caused by HCV remains the most common form of post-transfusion hepatitis, imposing a strong need for sensitive and specific diagnostic screening methods to identify potential blood donors and other persons who may be carriers of the virus and able to transmit the disease. Thus, accurate screening methods are needed to permit removal of contaminated blood and blood products from the blood supply with a high degree confidence.

The etiological agent HCV has been cloned and identified by several groups [Houghton *et al.*, EP 0318216, published 5/1989; Okamoto *et al.* (1990) *Jpn. J. Exp. Med.* 60:167; Houghton *et al.*, EP 0388232, published 9/1990; and Kato *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:9524; Arima *et al.* (1989a) *Gastroenterologia Japonica* 24:540; Reyes *et al.* (1990) *Science* 247:1335; Arima *et al.* (1989b) *Gastroenterologia Japonica* 24:545; Maeno *et al.* (1990) *Nucleic Acids Res.* 18:2685].

The HCV genome is about 10 kilobases (kb) in length and encodes a single polyprotein which is processed into structural and non-structural proteins. From the N terminus, the polyprotein includes the capsid and envelope proteins of the structural region and the NS-1 to NS-5

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proteins of the non-structural region.

While some of the antigenic regions of HCV have been identified, peptides and recombinant proteins from these regions exhibit a variable degree of sensitivity and selectivity in detection and diagnosis of HCV carriers.

Antigenic regions have been reported in the core, or capsid, protein [Wang, U.S. Patent No. 5,106,726; Hosein et al. (1991) Proc. Natl. Acad. Sci. USA 88:3647; Okamoto et al. (1990) Jap. J. Exp. Med. 60:223; Takahashi et al. (1992) J. Gen. Virol. 73:667; Kotwal et al. (1992) Proc. Natl. Acad. Sci. USA 89:4486]; in the envelope, NS-1, NS-2 and NS-3 proteins [Wang et al., EP 0468527, published Jan. 29, 1992]; NS-4 protein [Houghton (1989); Kuo et al. (1989) Science 244:362; U.S. Patent No. 5,106,726] and NS-5 protein [Maeno et al. (1990) Nucleic Acids Res. 18:2685; Wang (1992)].

In addition to HCV-derived antigens, there exist other HCV-associated antigens that appear to be encoded by a host cellular sequence. One such antigen, known as the GOR epitope, is reactive with sera from individuals who are PCR positive for HCV [Mishiro et al. (1990) Lancet 336:1400].

* Serological analysis has been used to map antigenic sites within certain HCV antigenic regions as described in Wang (1992) and U.S. Patent No. 5,106,726. These mapping studies employed synthetic peptides to screen well-characterized HCV serum panels and permitted identification of highly immunoreactive HCV antigenic sites.

The demonstration that synthetic peptides are efficacious for detection of antibodies to HCV has led to numerous studies using short synthetic peptides to characterize antigenic sites. For example, the Pepscan technique has been applied to the entire HCV-1 genome to begin elucidation of immunoreactive sites (Chien et al., WO 93/00365).



In the core protein, an immunodominant antigenic site located within amino acid residues 21-45 has been described [Nagayama et al. (1994) J. Med. Virol. 42:311-7; Siemoneit et al. (1994) Hybridoma 13:9-13; Ferroni et al. (1993) J. Clin. Microbiol. 31:1586-91; Ishida et al. (1993) J. Clin. Microbiol. 31:936-40]. [Note: the amino acid numbering system herein refers to the HCV-1 polyprotein numbering system]. Human monospecific antibodies to bind a peptide corresponding to core residues 33-50 [Akatsuka et al. (1993) Hepatology 18:503-10], and the binding site of a human monoclonal antibody directed against core was mapped to residues 34-45 [Cerino et al. (1993) J. Immunol. 151:7005-15]. Mouse monoclonal antibodies which bind to core residues 26-45 have been described [Gonzalez-Peralta et al. (1994) J. Hepatol. 20:143-7]. Another antigenic site at the N-terminus of core has been delineated at residues 1-18 by synthetic peptide studies [Sallberg et al. (1992a) J. Clin. Microbiol. 30:1989-94; Sallberg et al. (1992b) Immunol. Lett. 33:27-33]. Additional antigenic peptides were identified between these two sites at residues 11-28 [Sallberg, (1992a)] and 7-21 [Ferroni].

In NS-3, a conformational epitope was identified in the C-terminal 100 amino acids of the c-33c clone [Kink et al. WO 93/09253]. A human monoclonal antibody specific for this immunodominant antigenic site also bound to the C-terminus of NS-3 [Mondelli et al. (1994) J. Virol. 68:4829-36; Habets et al., WO 94/14974].

Synthetic peptide studies located two epitopes in the NS-4 protein at amino acid residues 1661-1708 and 1710-1728 [Simmonds et al. (1993) J. Clin. Microbiol. 31:1493-503]. Murine monoclonal antibodies bound to residues 1700-1705 [Gonzales-Peralta, supra], and a human monoclonal antibody directed against the NS-4 protein was reported to bind to residues 1688-1705 [Cerino et al. (1991) J. Immunol. 147:2692-6].



Many proteins from infectious agents, or at least sites on those proteins, exhibit strain variation at the sequence level. For example, at a given time or in a particular geographic locale, a particular antigen may contain one or more point mutations relative to an arbitrary prototype strain. Dynamic variation can thereby create an extremely complex antigenic profile at such a site for which sensitive or specific detection can be increasingly difficult as the site "drifts" further from the prototype. Accordingly, complex antigens can provide a simplified means to detect multiple strains.

For HCV, thus far, combinations of synthetic peptides from multiple proteins or regions of HCV have proven effective as diagnostic tests for HCV [Ishida; Wang (1992)]. However useful, such tests only represent sequences of a single strain. The known HCV sequence variability [Bukh et al. (1993) Proc. Natl. Acad. Sci. USA 90:8234-8] still poses a problem for sensitive antibody detection whether with peptide-based tests or with a recombinant protein-based test. A synthetic peptide test designated as KCL-163 based on a Japanese HCV strain proved both more sensitive and more specific than a C100-3 test (based on the HCV-1 strain) in a Japanese population [Kawano et al. (1991) Gastroenterol. Jpn. 26:218-20]. HCV-1 clones 5-1-1, C-33c, and C-22 in a RIBA format detected antibody from type II HCV-infected sera better than from type I HCV-infected sera [Alonso et al. (1994) J. Clin. Microbiol. 32:211-2], whereas the C-100-3 was more sensitive for type I than for type II serum [Nagayama et al. (1993) J. Clin. Invest. 92:1529-33].

Further, type-specific synthetic peptides from NS-4, can be used to discriminate among HCV genotypes [Simmonds]. However, a single amino acid in a core peptide (amino acids 101-108) of an HCV variant decreased reactivity with eight different HCV-1 sera [Sallberg (1992a)].



The present invention provides peptides which are sensitive and selective for the detection of HCV antibodies and can accommodate strain-related antigenic variation through the use of peptide libraries.

SUMMARY OF THE INVENTION

The present invention is directed to a peptide composition comprising at least one linear or at least one branched peptide represented by the formula

(peptide) - Y

(peptide)₂X

(peptide)₄X₂X

(peptide)₈X₄X₂X

(peptide)₁₆X₈X₄X₂X

wherein Y is an OH or NH₂ group on the carboxyl group of the C terminal amino acid of (peptide) and X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage. In accordance with this invention the peptide moiety, (peptide), is specifically immunoreactive with NANBH-associated antibodies, and comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5 to 34 (see Tables 1, 3, 5 and 7) or is one of the peptides of Formula I, II, III or IV containing the particular substitutions and degenerate positions as described in the "Detailed Description of the Invention".

Another aspect of the invention provides a method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an immunoeffective amount of the subject peptide composition in an immunoassay procedure, and particularly in an ELISA procedure, or a passive hemagglutination (PHA) assay. Immunoassays and kits for the detection and diagnosis of NANBH and HCV infection are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 graphically depicts the immunoreactivity



of a dilution series of human monoclonal antibody (B12.F8) specific for HCV core with Mixture A peptides (Example 6) and a commercially available HCV antibody detection kit (Ortho HCV 3.0; Ortho Diagnostic Systems, Inc.).

Fig. 2 graphically depicts the immunoreactivity of a dilution series of human monoclonal antibody (60H9[9]D10E6) specific for HCV NS-4 with Mixture A peptides (Example 6) and a commercially available HCV antibody detection kit (Ortho HCV 3.0).

Fig. 3 graphically depicts the immunoreactivity of a dilution series of human monoclonal antibody (CM3.B6) specific for HCV NS-3 with Mixture A peptides (Example 6) and a commercially available HCV antibody detection kit (Ortho HCV 3.0).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, extensive serological analysis has led to the refinement and further definition of immunoreactive peptides that are useful in the detection and diagnosis of HCV infection. Surprisingly it has been discovered that synthetic peptides containing multiple substitutions of natural and unnatural amino acids in a prototype HCV sequence are highly effective for detection and diagnosis of HCV infection. Likewise, these multiply-substituted peptides can contain degenerate positions with sequence variability to provide high selectivity and sensitivity for multiple strains of HCV. Accordingly, the preferred peptides of this invention are listed in Tables 1, 3, 5 and 7.

Each of the peptides in the Tables has a particular pattern of substitutions, deletions or degenerate positions in its peptide moiety relative to one of the four prototype sequences set forth below:

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



Arg-Thr,

(SEQ ID NO:1)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-
Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-
Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-
5 Lys-Ala-Leu-Gly-Leu,

IIH (SEQ ID NO:2)

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

VIIIE (SEQ ID NO:3)

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

(SEQ ID NO:4).

20 As used herein a position in a prototype
sequence wherein one amino acid is replaced by another is
termed a "substituted position". Similarly a position in
a prototype sequence wherein an amino acid thereof is not
present is termed a deleted position. Finally, a position
25 in a prototype sequence wherein a single amino acid is
replaced by a mixture of 2 or more amino acids during
peptide synthesis (i.e. as for library peptides described
below) is termed a degenerate position.

30 The peptides of this invention can be linear or
branched, and the invention includes polymers, conjugates
and mixtures of the peptides. Some peptides are hybrids
of one or more HCV antigens from different HCV proteins,
e.g. NS-4 and core.

35 Further some of the peptides contain degenerate
positions, i.e. these peptides represent "libraries" of



peptides as generally described in U.S. Serial No. 143,412, filed October 26, 1993 (the '412 application). ["Library peptides" as used herein are also referred to as "structured synthetic antigen libraries" or "SSAL" in the '412 application].

5 For library peptides, degenerate amino acid positions are those positions of known sequence variability, i.e., generated by strain to strain differences in HCV isolates. The degenerate positions thus contain any one of two or more amino acid residues.
10 The possible residues for a given position are determined from known sequence variations which occur at that position in the sequence of the peptide. The proportion of amino acids used during synthesis at a particular position can be represented by either the frequency at
15 which the given residues appear in the known variants, or by a simple equimolar distribution of the possible amino acids at that position. It should be noted that for this invention, not every sequence position susceptible to strain variation needs to be used.

20 As used herein a linear peptide can have from about 30 to about 100 amino acids, preferably about 40 to about 85 amino acids. However, the linear peptide can also consist of as few as 8 or 9 residues.

25 The peptides of the present invention are useful for the detection of antibodies to HCV in body fluids, and the diagnosis of HCV infection.

The subject peptides can have a few more amino acids, including unnatural amino acids, added to the terminal amino acids. For example, the sequence KKK (Lys-Lys-Lys) can be added to the amino terminus of any of
30 these peptides. For branched peptides, an M (methionine) residue can be placed at the carboxy terminus of the peptide moiety, i.e. between the peptide moiety and the branch structure. Similarly the peptides can have a
35 cysteine at the C terminus to facilitate using the thiol



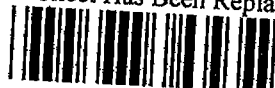
group of cysteine to form a covalent bond to an electrophilic group such as N^α-chloroacetyl-modified amino acid or a maleimide-derivatized α- or ε-NH₂ group of a lysine residue that is attached to the N-terminus of another peptide.

5 HCV is known to have frequent mutations. Several variant strains/isolates are known to exist, such as PT, J, J1 and J4 [Houghton, 1989; Okamoto, 1990; Houghton, 1990; and Kato, 1990] and it is expected that other variant strains also exist (Bukh). Adjustments for
10 conservative substitutions arising from strain variation can be made in the prescribed sequences, provided that the pattern of substituted, deleted, or degenerate positions in a given peptide are maintained. In this way, the peptides of this invention can accommodate the strain-to-
15 strain variation existing among different isolates of HCV via changes which do not affect antigenicity of the peptides.

Accordingly, the changes that are contemplated within the scope of the invention preserve the
20 immunoreactivity of the peptides with HCV antibodies but do not affect the pattern of substituted, deleted or degenerate positions. The changes can consist of substitutions, insertions, deletions or degeneracies at about 2 to about 5 positions, or alternatively at up to
25 about 10% of the positions in a peptide from Tables 1, 3, 5 or 7. Moreover, the contemplated changes are derived from known HCV strains.

Such peptides in accordance with this invention are synthesized and tested against an HCV serum panel to
30 determine the immunoreactivity of the peptide as described herein below.

The subject peptides can also be used to form conjugates, i.e., the peptides can be coupled directly or indirectly, by methods known in the art, to carrier
35 proteins such as bovine serum albumin (BSA), human serum



albumin (HSA), or to red blood cells or latex particles.

As used herein, natural amino acids are the 20 amino acids commonly found in proteins (i.e. alanine, aspartic acid, asparagine, arginine, cysteine, glycine, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan and valine). As used herein the natural amino acids also include the D- and L-forms of such amino acids.

As used herein "unnatural amino acids" include both D- and L- forms of any other amino acids whether found in a protein, whether found in nature or whether synthetically produced. Unnatural amino acids can include, but are not limited to, β -alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, gamma-amino butyric acid, homoserine, citrulline and the like.

The linear peptides of this invention are represented by the formula



wherein Y is -OH or -NH₂, and include mixtures, conjugates and polymers of these linear peptides. The peptides comprise at least one antigenic site which is specifically immunoreactive with antibodies against HCV.

The branched peptides of the present invention are represented by one of the formulae:



wherein X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage. Preferably X is lysine or a lysine analog such as ornithine. The amino acid analog can be an α -amino acid, a β -amino acid, or any other either natural or non-natural amino acid with two amino groups and one carboxyl group available for forming



peptide bonds. Preferred branched peptides of the invention are dimers, tetramers and octamers, especially those having a branching core structure composed of lysine, i.e. where X is lysine. Branched dimers and octamers are especially preferred.

5 The peptide moiety of the linear or branched peptides can vary in length from about 8 or 9 to about 100 amino acids residues. Preferably the peptide moieties contain about 9 to about 60 amino acid residues.

10 The preferred peptides of the present invention are provided in Tables 1, 3, 5 and 7.

Another aspect of this invention relates to peptide compositions having one or more of the peptides represented by formulas I, II, III or IV. Formula I peptides are those which substantially retain the frame of
15 the 81 amino acid prototype sequence of SEQ ID NO:4, which are immunoreactive with HCV NS-3 antibodies and which consist of the following substituted, deleted or degenerate positions:

20 position 3: norvaline, position 7: norvaline or Val:Ala:Thr:Phe:Tyr:Nvl at 3:3:1:1:1:1 ratio, position 8: valine or norvaline, position 16: norvaline, position 20: arginine, position 26: norleucine, position 33: norleucine, position 39: serine, position 46: norvaline, position 52:
25 alanine, position 60: serine, position 63: norleucine, position 68: serine, and position 74: norvaline.

Nvl is the abbreviation for norleucine. The positions referred to herein in Formulas I-IV are relative to those
30 in SEQ ID NOS:1-4, respectively.

Formula II peptides are those peptides which substantially retain the frame of the 61 amino acid prototype sequence of SEQ ID NO:3, which are immunoreactive with HCV core antibodies and which consist
35 of the following substituted or degenerate positions:



position 4: Pro:Gly at 9:1 ratio, position 8:
ornithine, position 10: Thr:Asn at 7:3 ratio,
position 16: lysine, position 21: norvaline,
position 24: hydroxyproline, position 30:
norvaline, position 36: valine or norleucine,
position 43: norleucine, position 45: leucine,
position 48: Thr:Pro at 9:1 ratio, position 52:
threonine or Thr:Ala:Glu:Lys at 1:1:1:1 ratio,
and position 57: hydroxyproline.

Formula III peptides are those peptides which
substantially retain the frame of the 47 amino acid
prototype sequence SEQ ID NO:2 followed by the 9 amino
acid prototype sequence of residues 21 to 29 of SEQ ID
NO:3, which are immunoreactive with HCV NS-4 or core
antibodies and which consist of the following substituted
or degenerate positions:

position 1: Ser:Asn at 8:2 ratio, position 2:
Gly:Asp:Gln at 8:1:1 ratio, position 3: Lys:Arg
at 6:4 ratio, position 4: Pro:Val:Ala at 8:1:1
ratio, position 5: threonine, position 6:
Ile:Val at 6:4 ratio, position 7: Ile:Val:Ala at
6:2:2 ratio, position 10: Arg:Lys at 8:2 ratio,
position 12: norvaline, position 15: Arg:Gln:Glu
at 3:5:2 ratio, position 16: Glu:Ala at 8:2
ratio, position 19: aspartate, position 22:
aspartate, position 24: Ser:Ala at 4:6 ratio,
position 25: Gln:Ser at 4:6 ratio, position 26:
His:Lys:Arg at 8:1:1 ratio, position 27: Leu:Ala
at 8:2 ratio, position 28: Pro:Ala at 8:2 ratio,
position 29: Tyr:Leu at 8:2 ratio, position 30:
norleucine or norvaline, position 31: aspartate,
position 32: Gln:Glu at 8:2 ratio, position 34:
Met:Gln at 8:2 ratio, position 35: Met:Gln:Arg
at 4:4:2 ratio, position 36: Leu:Met:Ile at
8:1:1 ratio, position 39: asparagine, position
40: Phe:Leu at 8:2 ratio, position 42: Gln:Ser



at 8:2 ratio, position 44: Ala:Ile at 8:2 ratio,
or position 45: valine, and position 55:
asparagine.

Formula IV peptides are those which
substantially retain the frame of the 44 amino acid
prototype sequence of SEQ ID NO:1, which are
immunoreactive with HCV NS-5 antibodies and which consist
of the following substituted or degenerate positions:

position 2: ornithine, position 8: Pro:Leu:Val
at 8:1:1 ratio, position 10: norvaline, position
12: Thr:Ser:Pro at 5:4:1 ratio, position 15:
Lys:Asp:Arg at 4:4:2 ratio, position 17:
glutamate, position 19: Glu:Val:Gln at 5:4:1
ratio, position 21: Pro:Ala at 9:1 ratio,
position 22: Val:Thr at 8:2 ratio, position 23:
norvaline, position 24: His:Leu:Ala at 8:1:1
ratio, position 27: Pro:Ala at 8:2 ratio,
position 30: Pro:Ser at 9:1 ratio, position 31:
hydroxyproline, position 32: Lys:Pro:Arg at
8:1:1 ratio, position 33: Ser:Ala:Lys: Gln at
4:4:1:1 ratio, position 34: Pro:Thr at 8:2
ratio, position 36: Val:Ile: Thr at 5:4:1 ratio,
position 37: hydroxyproline, position 41:
Lys:Arg at 4:6 ratio, position 42: Lys:Arg at
7:3 ratio, and position 44: Thr:Ala at 9:1
ratio.

The peptides represented by Formulas I, II, III
and IV can be linear or branched and the invention
includes polymers, conjugates and mixtures of these
peptides in accordance with those embodiments as described
herein.

As used herein, an "HCV NS-3 antibody" includes
an antibody or a serum or plasma sample containing
antibodies which immunoreact with or bind to peptide 4
(SEQ ID NO:4). As used herein an "HCV core antibody"
includes an antibody or a serum or plasma sample



containing antibodies which immunoreact with or bind to peptide VIIIE (SEQ ID NO:3). As used herein an "HCV NS-4 antibody" is an antibody or a serum or plasma sample containing antibodies which immunoreact with or bind to peptide IIH (SEQ ID NO:2). As used herein an "HCV NS-5 antibody" includes an antibody or a serum or plasma sample containing antibodies which immunoreact with or bind to peptide 1 (SEQ ID NO:1).

The peptide compositions of the present invention can be composed of one or more of the subject peptides. Preferably such compositions contain from one to 10 peptides, and even more preferably from one to four peptides.

In a preferred embodiment, the peptide compositions of the present invention include peptides 29, 33, 25, 19, 28 and 27, and preferably peptides 29, 33, 25 and 19. (The peptides are designated by their respective sequence listing identification numbers.)

Moreover, the peptide compositions of the present invention include mixtures of peptides. The effective ratio of peptides for diagnosing or detecting HCV present in peptide compositions containing mixtures of the subject peptides can be readily determined by one of ordinary skill in the art. Typically, these ratios range from about 1 to about 50 on a per weight basis of peptide.

A preferred peptide composition for diagnosis and detection of HCV infection is selected from a mixture of peptides known as Mixture A, which includes peptides 29, 33, 25 and 19; Mixture B which includes peptides 27, 33, 25 and 19; and mixture C which includes peptides 33, 25, 19 and 28. In a preferred embodiment Mixture A is coated at a weight ratio of 1:1:0.5:8.

To determine the efficacy of the subject peptides in detecting and diagnosing HCV antibodies, the peptides are tested for their immunoreactivity with specimens previously selected through the screening of



thousands of patient and normal sera for immunoreactivity with HCV. Such HCV-specific serum panels are commercially available and examples of serum panels and methods to select appropriate panel are provided in the Examples.

The strategy for serological validation depends on the expected characteristics of the target antigenic sites. For example, universal immunodominant sites, such as the gp41 transmembrane peptide of HIV-1, can be screened by a single representative serum sample from a patient known to be infected with the virus. Antigenic sites that are not recognized by all infected individuals, or those for which antibody is produced late or only transiently, must be screened by large panels of sera. While both methods of screening can be employed in the present invention to refine the antigenic analysis for HCV using the subject peptides, the latter method is particularly useful in assessing the subject peptides for superior selectivity and sensitivity.

The identification of the antigenic sites is also dependent on the panel of sera used. The more closely the panel represents the population most likely to be seropositive for a site, the greater the chance that the antigenic site will be identified and thoroughly mapped. Hence, to extend the range of reactivity of an assay comprised of previously identified antigenic sites or epitopes, a large number of samples from individuals at risk of infection but seronegative against known antigens or epitopes should be employed for screening.

The process of "serological validation" is particularly difficult when the antigenic site to be identified elicit antibodies only in a subpopulation of an infected patient group. When such antigens become targets for identification, special attention must be paid to synthetic peptides which show very weak reactivity.

In this regard, the low background absorbance of synthetic peptides, especially peptides with unnatural



amino acids, allows for the precise detection of weak reactivities. In some cases, absorbances of 50 mAU versus background reading are of sufficient significance and can lead to the identification of important antigenic sites through successive refinement of the amino acid sequence of a peptide. With good laboratory practices, consistent and reliable results can be obtained when working in the range of absorbances below 200-300 mAU.

The peptides can be readily synthesized using standard techniques, such as the Merrifield method of synthesis [Merrifield (1963) J. Am. Chem. Soc. 85:2149-2154].

Another problem which can be minimized by using peptides rather than recombinantly expressed proteins (or peptides) is the rate of false positive results caused by the presence of antigenic material co-purified with the HCV recombinant. For example, certain normal individuals have antibodies to Escherichia coli or yeast proteins which are cross reactive with the antigenic materials from the expression system used in recombinant-based diagnostic tests. Sera from such normal individuals can show a false positive reaction in such immunoassays which false reaction is eliminated in immunoassays of the present invention.

Moreover, because the peptide compositions of the present invention are synthetically prepared, the quality can be controlled and as a result, reproducibility of the test results can be assured. Also, since very small amounts of a peptide are required for each test procedure, and because the expense of preparing a peptide is relatively low, the cost of screening body fluids for antibodies to HCV and diagnosis of HCV infection is relatively low.

As a further advantage, the use of library peptides provides the assay with a broader spectrum of reactivity against a greater number of HCV strains. In



essence, degenerate positions of the library peptides represent an "open" diagnostic tool which accommodates the strain-specific sequence variation (and concomitant variation in antibody specificity) arising from multiple strains of HCV. Hence, library peptides enhance the ability of the antigenic peptides to react with antibodies specific for a wider range of HCV variants.

The peptides and peptide compositions prepared in accordance with the present invention can be used to detect and diagnose HCV infection by using them as the test reagent in an enzyme-linked immunoadsorbent assay (ELISA), an enzyme immunodot assay, a passive hemagglutination assay (e.g., PHA test), an antibody-peptide-antibody sandwich assay, a peptide-antibody-peptide sandwich assay, or other well-known immunoassays. In accordance with the present invention, any suitable immunoassay can be used with the subject peptides. Such techniques are well known to the ordinarily skilled artisan and have been described in many standard immunology manuals and texts, see for example, by Harlow et al. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 726 pp. In a preferred embodiment, the immunoassay is an ELISA using a solid phase coated with the peptide compositions of the present invention. ELISA techniques are well known in the art. In another preferred embodiment the immunoassay is a PHA assay.

The immunoassays of the present invention are used to screen body fluids and tissues for the presence of HCV reactive antibody and thereby aid the practitioner in diagnosis of HCV infection. The body fluids which can be screened include blood and blood fractions (e.g. plasma and serum), saliva, or any other fluid which is suspected of containing antibodies against HCV.

Another aspect of the present invention is directed to a kit for the detection of HCV antibodies or



diagnosis of HCV infection in mammalian body fluids (e.g. serum, tissue extracts, tissue fluids), in vitro cell culture supernatants, and cell lysates. The kit can be compartmentalized to receive a first container adapted to contain one or more of the peptides (i.e. a peptide composition) of this invention.

Preferably the kit of this invention is an ELISA or a PHA test kit for detection of HCV antibodies or diagnosis of HCV infection. For an ELISA test kit, the kit contains (a) a container (e.g., a 96-well plate) having a solid phase coated with one of the subject peptide compositions; (b) a negative control sample; (c) a positive control sample; (d) specimen diluent and (e) antibodies to human IgG, which antibodies are labelled with a reporter molecule. If the reporter molecule is an enzyme, then the kit also contains a substrate for said enzyme.

In an exemplified use of the subject kit, a sample to be tested is contacted with a mammalian body fluid, diluted in sample diluent if necessary, for a time and under conditions for any antibodies, if present, to bind to the peptide contained in the container. After removal of unbound material (e.g. by washing with sterile phosphate buffered saline), the secondary complex is contacted with labelled antibodies to human IgG. These antibodies bind to the secondary complex to form a tertiary complex and, since the second antibodies are labeled with a reporter molecule, when subjected to a detecting means, the tertiary complex is detected. The reporter molecule can be an enzyme, radioisotope, fluorophore, bioluminescent molecule, chemiluminescent molecule, biotin, avidin, streptavidin or the like. For ELISA the reporter molecule is preferably an enzyme.

The examples serve to illustrate the present invention and are not to be used to limit the scope of the invention.



EXAMPLE 1

ELISA Assay Method

5 The wells of 96-well plates were coated separately for 1 hour at 37°C with 5 µg/ml of peptide using 100 µL per well in 10mM NaHCO₃ buffer, pH 9.5 unless noted otherwise.

10 The peptide-coated wells were incubated with 250 µL of 3% by weight of gelatin in PBS in 37°C for 1 hour to block non-specific protein binding sites, followed by three washes with PBS containing 0.05% by volume of TWEEN 20 and dried. The test specimens containing HCV antibody positive patient sera were diluted 1:20 volume to volume with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20. 100 µL of the diluted specimens were added to each of the wells and
15 allowed to react for 30 minutes at 37°C.

The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti-human IgG was used as a second antibody tracer to bind
20 with the HCV antibody-peptide antigen complex formed in positive wells. 100 µL of peroxidase labeled goat anti-human IgG at a dilution of 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37°C for another 15 minutes.

25 The wells were washed six times with 0.05% by volume TWEEN 20 PBS to remove unbound antibody and reacted with 100 µL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0. This
30 substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 µL of 1.0M H₂SO₄ and the A_{492nm} measured.



EXAMPLE 2

Immunoreactivity of NS-3-Reactive Peptides

Wells of 96-well plates were coated separately as in Example 1 for 1 hour at 37°C with each of the indicated 17 peptides (Table 2, the sequences of which are provided in Table 1). The peptides were tested on a selected panel of sera reactive with an immunodominant conformational epitope from the NS-3 protein but not with linear epitopes from the same region. Panel members were selected as follows: HCV-seropositive plasma specimens from a commercial source (North American Biologicals, Inc) were screened for reactivity with the synthetic Peptide 4 (SEQ ID NO:4) containing conformational and linear epitopes [Mondelli (1994)]. Specimens that were reactive with Peptide 4 were further screened on Pep18 [Wang (1992)]. Pep18 contains linear epitopes, but does not exhibit the conformational epitope. The selected serum panel then constituted those specimens that reacted with Peptide 4 but not with Pep18.

The sums of the EIA absorbance readings at 492 nm are tabulated for each peptide (Table 2). The results show that the peptides had reactivity for a partially or fully preserved conformational epitope present in the Peptide 4 (36-103% as reactive).

EXAMPLE 3

Immunoreactivity of Core-Reactive Peptides

The immunoreactivity of peptides 22 and 26 octamer (Table 3) was determined with a group of known HCV serum samples from HCV Panel 3 containing antibodies against core in the ELISA assay format as described in Example 1. The results are shown in Table 4. The absorbances of both peptides 22 and 26 were greater than that of VIIIE (SEQ ID NO:3) on samples 3-3, 3-8 and 3-26. Peptide 26 was stronger than VIIIE on sample 3-39, and peptide 22 octamer was stronger than VIIIE on samples 3-1 and 3-41.

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EXAMPLE 4

Immunoreactivity of NS-4-Reactive Peptides and
NS-4/Core-Reactive Hybrid Peptides

The immunoreactivity of peptides 3KIIH, 27, 28,
29, and 30 (Table 5) was determined with a panel of known
HCV sera from HCV panel 3 containing antibodies to the NS-
4 protein in the ELISA assay format as described in
Example 1. The results are shown in Table 6. All of the
peptides showed stronger reactivity than the prototype
HCV-1 strain peptide 3KIIH.

EXAMPLE 5

Immunoreactivity of NS-5-Reactive Peptides

The immunoreactivity of NS-5-reactive peptides
(Table 7) with a panel of known HCV sera from HCV Panel 3
was assessed in the ELISA assay format as described in
Example 1. The results (Table 8) indicate that these
peptides exhibited similar immunoreactivity. All three of
the library peptides (31, 32 and 33) containing mixtures
of amino acids at fixed positions displayed
immunoreactivity comparable to or greater than peptide 34
which was not a library peptide.

EXAMPLE 6

Detection of HCV Antibodies by Peptide Mixtures

Mixture A containing peptides 25, 29, 33 and 19,
the latter peptide conjugated to BSA, was coated at a
weight ratio of 2:2:0.5:8 ($\mu\text{g/mL}$) and compared to Mixture
B containing peptides 25, 27 octamer, 33 and 19, the
latter peptide conjugated to BSA, coated at a weight ratio
of 2:2:0.5:8 ($\mu\text{g/mL}$) in the ELISA assay format described
in Example 1.

Peptide 19 was conjugated to BSA using m-
maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) in
accordance with manufacturer's recommendations (Pierce
Chemical Co.) or Kitagawa et al (1976) J. Biochem. 79:233-
236.

To assess the sensitivities of Mixtures A and B,



the mixtures were assayed with a specially-selected serum panel containing panel members that had specific immunoreactivity for the HCV core, NS-3, NS-4 or NS-5 regions. This panel was prepared by screening sera against peptides VIIIE (SEQ ID NO:3) to identify core-specific sera, against peptide IIH (SEQ ID NO:2) to identify NS-4-specific sera, against Pep11 (Wang, 1992) to identify NS-5-specific sera and against Peptide 4 (SEQ ID NO:4) to identify NS-3-specific sera. The so-identified sera were then diluted with normal human sera to exhibit weak to moderate reactivity on these same peptides (i.e., to provide an absorbance at 492 nm ranging from 0.3 to 2.0). The diluted samples then constituted the special sensitivity panel (Table 9) used for assay on Mixtures A and B.

The two mixtures had comparable sensitivity (Table 9).

EXAMPLE 7

Mixture A (Example 6) was tested for sensitivity with a dilution series of human monoclonal antibodies specific for a) the core protein of HCV, B12.F8 [Cerino (1993)], b) the NS-4 protein of HCV, 60H9[9]D10E6 [Cerino (1991)] and c) the NS-3 protein conformational epitope, CM3.B6 [Mondelli (1994)]. The same dilution series was tested in parallel with a commercially available anti-HCV kit (Ortho HCV 3.0) composed of a mixture of recombinant proteins from the core, NS-3, NS-4 and NS-5 regions. The analytical sensitivity for detection of antibodies to all three regions was 2- to 8- fold higher using the Mixture A peptides over that obtained with the recombinant proteins of the commercially available kit (Figs. 1-3).

EXAMPLE 8

A total of 1034 samples that were confirmed positive for HCV antibodies by PCR analysis [Wang et al. (1992) Gastroenterology 103:609] or by Chiron* RIBA* HCV 2.0 Strip Immunoblot Assay (*TM; Ortho Diagnostic Systems,



Inc, Raritan, NJ) were assayed on Mixture A ELISA in accordance with Examples 1 and 6. These samples were collected from NANBH patients with chronic or acute hepatitis, hemophiliacs, multiply transfused patients, HCV-infected patients undergoing seroconversion, and blood donors identified as previously infected with HCV. All 1034 confirmed-positive samples were positive on the Mixture A assay with a mean signal/cutoff ratio of 10.

A collection of 3154 random blood donor samples was likewise tested on the Mixture A ELISA. The specificity of the assay was greater than 99.5% in blood donor populations. The mean signal/cutoff ratio of the samples was 0.3. Thus the confirmed positive samples had a mean signal/cutoff ratio that was 33-fold higher than that of the random blood donor samples.

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Table 1
NS-3-Reactive Peptides

SEQ. ID	SEQUENCE ^{a, b}
5	KKKKAIPLEVIKGGKHLIFCHSKKKCDELAAKLVALGINAVAYYRGIDVSIPTSGDPTALMTGYTGDFDSVIDC
6	KAVPLEVVKGGRHLIVCHTKKKCDELAAKLVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDC
7	KAVPLEVVKGGRHLIVCHTKKKCDEZAALVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV
8	KAVPLEVVKGGRHLIVCHAKKKCDEZAALVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV
9	KAVPLE*VKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV
10	KAVPLE*VKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV
11	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV
12	KAVPLE*VKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFESVIDCNTCV
13	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYTGDFDSVIDCNTCV
14	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYTGDFDSVIDCNTCV
15	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
16	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
17	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
18	KAVPLE*VKGGRHLIVCHSKKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
19	KAVPLE*VKGGRHLIVCHSRKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
20	KAVPLE*VKGGRHLIVCHSRKKCDEZAALVALGINAVAYYRGIDVSIPTAGDVVVVATDALMTGYSGDFDSVIDCNTCV
21	KKKKAIPLEVIKGGRHLIFCHSRRCDELAAKLVALGINAVAYYRGIDVSIPTSGDVVVVATDALMTGYTGDFDSVIDCNTCV

^a The sequences in this and Tables 3, 5 and 7 are provided in standard one letter amino acid codes. For unnatural amino acids, the codes are v, norvaline; p, hydroxyproline; o, ornithine and z, norleucine.

^b The position represented by * is a library position having the amino acid distribution V₉A₁T₁F₁X₁Y₁.



Table 2

Immunoreactivity of NS-3-Reactive Peptides

Serum		4	5	6	7	8	9	10	11
5	HCV3-3	0.448	0.079	0.376	0.249	0.231	0.362	0.266	0.318
	HCV3-9	0.737	0.043	0.802	0.598	0.232	0.710	0.514	0.083
	HCV3-14	1.542	1.785	0.915	0.634	0.437	1.044	0.784	0.502
	HCV3-18	0.860	0.036	0.340	0.197	0.222	0.365	0.258	0.293
	HCV3-19	1.725	1.804	0.663	0.463	0.504	0.855	0.715	0.318
10	HCV3-25	1.640	2.369	1.424	0.452	0.734	1.127	0.850	0.888
	HCV3-27	1.184	0.931	0.921	0.593	0.316	0.978	0.702	0.739
	HCV3-32	0.632	0.564	0.181	0.146	0.131	0.363	0.331	0.106
	HCV3-36	1.968	0.057	0.737	0.865	0.319	1.297	1.332	0.409
	HCV3-39	2.245	2.367	1.624	0.713	1.561	1.630	1.464	1.246
15	Sum*	12.981	10.035	7.983	4.910	4.687	8.731	7.216	4.902
	% ^b	100	77	61	38	36	67	56	38

Serum		12	13	14	15	16	17	18	19	21
20	V3-3	0.151	0.374	0.191	0.431	0.182	0.312	0.172	0.517	0.233
	V3-9	0.431	0.781	0.299	0.683	0.380	0.539	0.783	1.306	0.365
	V3-14	0.151	0.966	0.604	1.050	0.336	1.028	0.904	1.672	0.354
	V3-18	0.055	0.478	0.200	0.288	0.069	0.422	0.372	0.933	0.098
	V3-19	0.396	0.681	0.419	0.622	0.378	0.794	0.709	1.307	0.388
25	V3-25	0.946	1.144	0.815	1.085	0.747	0.876	0.798	1.260	0.628
	V3-27	0.384	0.994	0.656	1.097	0.435	0.863	1.023	1.706	0.313
	V3-32	0.201	0.679	0.127	0.473	0.091	0.375	0.460	0.959	0.076
	V3-36	0.971	0.704	0.457	1.415	0.494	0.904	1.334	2.078	0.810
	V3-39	1.625	1.345	1.411	1.555	0.810	1.330	1.032	1.611	1.069
30	m ^a	5.311	8.146	5.179	8.699	3.922	7.443	7.587	13.349	4.334
	% ^b	41	63	40	67	30	57	58	103	33

^aSum of the above absorbances at 492nm.

^bPercentage of immunoreactivity relative to Peptide 4.



Table 3

Core-Reactive Peptide

SEQ. ID	SEQUENCE
22	VKPPGGGQIM-OCT
23	KKKIPKPNOKT KENTQRRPNDVKPPGGGNTVGGVYLTPRRGPRZGLRATRKTTERSQPRGR-DIM
24	KKKSTIP,KPQOKT,KRNTNKRPDVKPPGGGQIVGGVYLTPRRGPRZGLRAT,PKTTERSQPRGR G ₁ N ₃ P ₁
25	KKKSTIP,KPQOKT,KRNTNKRPDVKPPGGGQIVGGVYLTPRRGPRZGLRAT,PKTTERSQPRGR G ₁ N ₃ P ₁

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Table 4

Immunoreactivity of Core-Reactive Peptides

Serum	VIIIE	26	22 Octamer
5 HCV3-1	3.273	3.600	3.702
HCV3-3	2.885	3.173	3.161
HCV3-8	1.137	1.517	1.860
HCV3-26	2.979	3.277	3.233
HCV3-39	1.032	1.329	0.486
10 HCV3-41	3.268	3.595	3.614
Sum ^a	14.574	16.491	16.056
% ^b	100	113	110

^a Sum of the above absorbances at 492nm.

^b Percentage of immunoreactivity relative to peptide VIIIE.

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Table 5

NS-4-Reactive Peptides

SEQ. ID	SEQUENCE
27	IIPDREVLVM-OCT
28	KKKSGKPTIIPDREVLVREFDDMEDCSQHLPYZDQGMIAENFKQKAVGL
29	KKKS ₂ G ₂ K ₂ P ₂ TI ₂ IPDR ₂ EVLYR ₂ E ₂ FDDMEDCS ₂ QH ₂ I ₂ P ₂ Y ₂ ZDQGM ₂ L ₂ AENF ₂ KQ ₂ KA ₂ VGLVKFPGGNI N ₂ D ₂ R ₂ V ₂ V ₂ V ₂ K ₂ Q ₂ A ₂ A ₂ S ₂ K ₂ A ₂ A ₂ L ₂ E ₂ Q ₂ Q ₂ M ₂ L ₂ S ₂ I ₂ Q ₂ A ₂ A ₂ A ₂ E ₂ R ₂ I ₂
30	KKKS ₂ G ₂ K ₂ P ₂ TI ₂ IPDR ₂ EVLYR ₂ E ₂ FDDMEDCS ₂ QH ₂ I ₂ P ₂ Y ₂ VDQGM ₂ L ₂ AENF ₂ KQ ₂ KA ₂ VGLVKFPGGNI N ₂ D ₂ R ₂ V ₂ V ₂ V ₂ K ₂ Q ₂ A ₂ A ₂ S ₂ K ₂ A ₂ A ₂ L ₂ E ₂ Q ₂ Q ₂ M ₂ L ₂ S ₂ I ₂ Q ₂ A ₂ A ₂ A ₂ E ₂ R ₂ I ₂

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Table 6

Immunoreactivity of NS-4-Reactive Peptides						
	Serum	3KIIH	27	28	29	30
5	HCV3-5	1.820	1.276	2.575	2.670	2.503
	HCV3-16	2.002	1.931	2.262	2.702	2.112
	HCV3-17	2.091	3.268	2.566	3.000	2.486
	HCV3-19	2.386	2.343	2.598	3.000	2.381
10	HCV3-24	2.106	3.497	2.699	2.401	2.210
	HCV3-25	1.813	1.794	2.388	2.266	2.040
	HCV3-33	1.361	1.144	2.055	2.327	2.147
	HCV3-36	1.383	1.480	2.453	2.277	2.374
15	HCV3-39	1.663	1.679	1.915	1.885	1.682
	HCV3-41	1.896	3.863	3.000	2.473	3.000
Sum ^a		18.1	22.275	24.511	25.001	22.935
% ^b		100	120	132	135	124

20^a Sum of the above absorbances at 492nm

^b Percentage of immunoreactivity relative to 3KIIH

25

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Table 7
NS-5-Reactive peptides

SEQ. ID	SEQUENCE
33	DYNPP ₈ LVET ₃ WKK ₄ PEYE ₃ PP ₃ V ₈ HH ₆ GCP ₈ LP ₈ PK ₅ S ₈ PV ₈ PPRK ₄ K ₂ RT ₁ L ₁ S ₄ D ₄ V ₄ A ₁ T ₂ L ₁ A ₂ S ₁ P ₁ A ₁ T ₂ I ₄ R ₂ R ₃ A ₁ V ₁ P ₁ R ₂ Q ₁ T ₁ A ₁ Q ₁
34	PDYNPP ₈ LVET ₃ WKK ₄ PEYE ₃ PP ₃ V ₈ HH ₆ GCP ₈ LP ₈ PK ₅ S ₈ PV ₈ PPRK ₄ K ₂ RT ₁ L ₁ S ₄ D ₄ V ₄ A ₁ T ₂ L ₁ A ₂ S ₁ P ₁ A ₁ T ₂ I ₄ R ₂ R ₃ A ₁ V ₁ P ₁ R ₂ Q ₁ A ₁ Q ₁
35	AOPDYNPP ₈ LVET ₃ WKK ₄ PEYE ₃ PP ₃ V ₈ HH ₆ GCP ₈ LP ₈ PK ₅ S ₈ PV ₈ PPRK ₄ K ₂ RT ₁ L ₁ S ₄ D ₄ V ₄ A ₁ T ₂ L ₁ A ₂ S ₁ P ₁ A ₁ T ₂ I ₄ R ₂ R ₃ A ₁ V ₁ P ₁ R ₂ Q ₁ A ₁ Q ₁
36	PLVETWKKPEYEPV ₈ HGCP ₈ LP ₈ PK ₅ PPV ₈ PPRK ₄ K ₂ RT ₁ -DIM

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Table 8

Immunoreactivity of NS-5-Reactive Peptides				
Serum	31	32	33	34
HCV3-1	1.974	2.155	2.215	2.097
5 HCV3-5	0.802	0.906	0.937	0.687
HCV3-17	1.946	2.183	2.339	2.742
HCV3-19	1.920	2.023	2.051	1.781
HCV3-33	1.191	1.385	1.583	0.256
HCV3-41	1.254	0.806	1.885	1.877
10 Sum ^a	9.087	9.458	11.01	9.440
% ^b	96	100	117	100

^a Sum of the above absorbances at 492nm.

^b Percentage of immunoreactivity relative to Peptide 32.

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Table 9

Sensitivity of Peptide Mixtures

Sample ^a	Mixture A		Mixture B		
	Abs	Ratio ^b	Abs	Ratio ^b	
5	Core-1	1.099	2.57	1.085	2.67
	Core-2	2.444	5.72	2.210	5.44
	Core-3	1.646	3.85	1.747	4.30
	NS3-1	0.664	1.55	0.642	1.58
	NS3-2	1.557	3.65	1.757	4.33
	NS4/NS3-1	1.902	4.45	1.819	4.48
	NS4-1	1.404	3.29	0.658	1.62
10	Neg-1	0.163	0.38	0.180	0.44
	Neg-2	0.170	0.40	0.191	0.47
	Neg-3	0.136	0.32	0.152	0.37

^a Panel members selected as described in Example 7, Neg is normal human serum known to be negative for HCV antibodies

^b Ratio is the absorbance over the cutoff value. Cutoff value for Mixture A was 0.427 and for Mixture B was 0.406.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Hosein, Barbara
Wang, Chang Yi
- 5 (ii) TITLE OF INVENTION: Peptides Effective for
Diagnosis and Detection of Hepatitis C Infection
- (iii) NUMBER OF SEQUENCES: 34
- (iv) CORRESPONDENCE ADDRESS:
- 10 (A) ADDRESSEE: Morgan & Finnegan, L.L.P
(B) STREET: 345 Park Avenue
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10154
- (v) COMPUTER READABLE FORM:
- 15 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version
#1.25
- (vi) CURRENT APPLICATION DATA:
- 20 (A) APPLICATION NUMBER:
(B) FILING DATE: 23 October 1995
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION
- (A) APPLICATION NUMBER: 08/333,573
(B) FILING DATE: 01 November 1994
- 25 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Maria C.H. Lin
(B) REGISTRATION NUMBER: 29,323
(C) REFERENCE/DOCKET NUMBER: 1151-4118PC
- (ix) TELECOMMUNICATION INFORMATION:
- 30 (A) TELEPHONE: (212)758-4800
(B) TELEFAX: (212)751-6849

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
- 35 (A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Thr
 1 5 10
 Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val Val His
 15 20
 5 Gly Cys Pro Leu Pro Pro Pro Lys Ser Pro Pro Val
 25 30 35
 Pro Pro Pro Arg Lys Lys Arg Thr
 40

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 47 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val
 1 5 10
 Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ser
 15 20
 20 Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu
 25 30 35
 Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu
 40 45

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 61 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg
 1 5 10
 Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe Pro
 15 20
 35

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Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu
 25 30 35
 Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr
 40 45
 Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg
 50 55 60
 5 Arg

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg
 1 5 10
 His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp
 15 20
 Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35
 20 Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 25 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 75 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

35

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Lys Lys Lys Lys Ala Ile Pro Leu Glu Val Ile Lys
 1 5 10
 Gly Gly Lys His Leu Ile Phe Cys His Ser Lys Lys
 15 20
 Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala Leu
 25 30 35
 5 Gly Ile Asn Ala Val Ala Tyr Tyr Lys Gly Leu Asp
 40 45
 Val Ser Val Ile Pro Thr Ser Gly Asp Thr Asp Ala
 50 55 60
 Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val
 65 70
 Ile Asp Cys
 75

10

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- 15
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
 - (B) LOCATION: 3
 - (D) OTHER INFORMATION: /note= "Norvaline"
- 20
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
 - (B) LOCATION: 7
 - (D) OTHER INFORMATION: /note= "Norvaline"
- 25
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
 - (B) LOCATION: 16
 - (D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Ala Xaa Pro Leu Glu Xaa Val Lys Gly Gly Arg
 1 5 10
 His Leu Ile Xaa Cys His Thr Lys Lys Lys Cys Asp
 15 20
 Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35
 Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45

35

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Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 Ser Val Ile Asp Cys
 75

5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS: /
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"

15

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"

20

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /note= "Norleucine"

25

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /note= "Norvaline"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Ala Xaa Pro Leu Glu Xaa Val Lys Gly Gly Arg
 1 5 10
 His Leu Ile Xaa Cys His Thr Lys Lys Lys Cys Asp
 15 20
 Glu Xaa Ala Ala Lys Leu Xaa Ala Leu Gly Ile Asn
 25 30 35

35

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Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 5 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /note= "Norleucine"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 38
 (D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Ala Xaa Pro Leu Glu Xaa Val Lys Gly Gly Arg
 1 5 10
 His Leu Ile Xaa Cys His Ala Lys Lys Lys Cys Asp
 15 20
 35

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Glu Xaa Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35
 Ala Xaa Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Ala Thr
 50 55 60
 5 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

(2) INFORMATION FOR SEQ ID NO:9:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 20 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note=
 "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 38
 25 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 46
 (D) OTHER INFORMATION: /note= "Norvaline"

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
 1 5 10
 His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp
 15 20
 Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35



Ala Xaa Ala Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 5 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note=
 "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
 1 5 10
 25 His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp
 15 20
 Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35
 Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 30 Asp Ala Leu Met Thr Gly Tyr Thr Gly Glu Phe Asp
 65 70
 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80



(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "Norvaline"

10

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

15

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 16
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 26
- (D) OTHER INFORMATION: /note= "Norleucine"

20

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 46
- (D) OTHER INFORMATION: /note= "Norvaline"

25

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 74
- (D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

30

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
1 5 10
His Leu Ile Xaa Cys His Ser Lys Lys Lys Cys Asp
15 20
Glu Xaa Ala Ala Lys Leu Val Ala Leu Gly Ile Gln
25 30 35
Ala Val Ala Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
40 45

35

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Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr
 50 55 60
 Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp
 65 70
 Ser Xaa Ile Asp Cys Asn Thr Cys Val
 75 80

5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "Norvaline"

15

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

20

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 63
- (D) OTHER INFORMATION: /note= "Norleucine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

25

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
 1 5 10
 His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp
 15 20
 Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
 25 30 35
 Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
 40 45
 Ile Pro Thr Ser Gly Asp Val Val Val Ala Thr
 50 55 60
 Asp Ala Xaa Met Thr Gly Tyr Thr Gly Asp Phe Glu
 65 70
 Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

35

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "Norvaline"

10

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

15

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 16
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 26
- (D) OTHER INFORMATION: /note= "Norleucine"

20

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 46
- (D) OTHER INFORMATION: /note= "Norvaline"

25

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 50
- (D) OTHER INFORMATION: /note= "Hydroxyproline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 63
- (D) OTHER INFORMATION: /note= "Norleucine"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
1 5 10

35

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```

His Leu Ile Xaa Cys His Ser Lys Lys Lys Cys Asp
      15                20
Glu Xaa Ala Ala Lys Leu Val Ala Leu Gly Ile Asn
  25      30                35
Ala Val Ala Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
      40                45
5  Ile Pro Thr Ala Gly Asp Val Val Val Val Ala Thr
      50                55                60
Asp Ala Xaa Met Thr Gly Tyr Thr Gly Asp Phe Asp
      65                70
Ser Val Ile Asp Cys Asn Thr Cys Val
      75                80

```

10 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"

20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note=
 "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

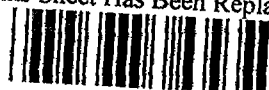
25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"

30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 35
 (D) OTHER INFORMATION: /note= "Norleucine"

35 (ix) FEATURE:

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(A) NAME/KEY: Modified-site
(B) LOCATION: 44
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

5 (A) NAME/KEY: Modified-site
(B) LOCATION: 63
(D) OTHER INFORMATION: /note= "Norleucine"

(xi) SEQUENCE DESCRIPTION: SEQ /ID NO:14:

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
1 5 10
10 His Leu Ile Xaa Cys His Ser Lys Lys Lys Cys Asp
15 20
Glu Xaa Ala Ala Lys Leu Val Ala Leu Gly Xaa Asn
25 30 35
Ala Val Ala Tyr Tyr Arg Gly Xaa Asp Val Ser Val
40 45
Ile Pro Thr Ala Gly Asp Val Val Val Val Ala Thr
50 55 60
15 Asp Ala Xaa Met Thr Gly Tyr Thr Gly Asp Phe Asp
65 70
Ser Val Ile Asp Cys Asn Thr Cys Val
75 80

(2) INFORMATION FOR SEQ ID NO:15:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

30

(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note=
"V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

(ix) FEATURE:

35

(A) NAME/KEY: Modified-site

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(B) LOCATION: 16
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 26
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 46
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 63
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 74
(D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Lys	Ala	Xaa	Pro	Leu	Glu	Val	Val	Lys	Gly	Gly	Arg
1				5					10		
His	Leu	Ile	Xaa	Cys	His	Ser	Lys	Lys	Lys	Cys	Asp
	15					20					
Glu	Xaa	Ala	Ala	Lys	Leu	Val	Ala	Leu	Gly	Ile	Asn
25				30					35		
Ala	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Xaa	Ser	Val
		40				45					
Ile	Pro	Thr	Ala	Gly	Asp	Val	Val	Val	Val	Ala	Thr
	50				55					60	
Asp	Ala	Xaa	Met	Thr	Gly	Tyr	Ser	Gly	Asp	Phe	Asp
			65					70			
Ser	Xaa	Ile	Asp	Cys	Asn	Thr	Cys	Val			
	75					80					

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note=
 "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /note= "Norleucine"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /note= "Norleucine"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 46
 (D) OTHER INFORMATION: /note= "Norvaline"
- 20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 63
 (D) OTHER INFORMATION: /note= "Norleucine"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 74
 (D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

30	Lys	Ala	Xaa	Pro	Leu	Glu	Val	Val	Lys	Gly	Gly	Arg
	1				5				10			
	His	Leu	Ile	Xaa	Cys	His	Ser	Lys	Lys	Lys	Cys	Asp
		15				20						
	Glu	Xaa	Ala	Ala	Lys	Leu	Val	Ala	Xaa	Gly	Ile	Asn
	25				30				35			
	Ser	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Xaa	Ser	Val
35			40					45				

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Ile Pro Thr Ala Gly Asp Val Val Val Ala Ser
50 55 60
Asp Ala Xaa Met Thr Gly Tyr Ser Gly Asp Phe Asp
65 70
Ser Xaa Ile Asp Cys Asn Thr Cys Val
75 80

5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS: /

- (A) LENGTH: 81 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "Norvaline"

15

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

20

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 16
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 26
- (D) OTHER INFORMATION: /note= "Norleucine"

25

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 33
- (D) OTHER INFORMATION: /note= "Norleucine"

30

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 46
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site

35

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(B) LOCATION: 63
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 74
(D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
 1          5          10
His Leu Ile Xaa Cys His Ser Lys Lys Lys Cys Asp
10          15          20
Glu Xaa Ala Ala Lys Leu Val Ala Xaa Gly Ile Asn
25          30          35
Ala Ile Ala Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
          40          45
Ile Pro Thr Ala Gly Asp Val Val Val Val Ala Ser
          50          55          60
15 Asp Ala Xaa Met Thr Gly Tyr Ser Gly Asp Phe Asp
          65          70
Ser Xaa Ile Asp Cys Asn Thr Cys Val
          75          80
```

(2) INFORMATION FOR SEQ ID NO:18:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Norvaline"

30 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note=
"V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

35 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 16

This Sheet Has Been Replaced



(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 26

(D) OTHER INFORMATION: /note= "Norleucine"

5

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 33

(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 46

(D) OTHER INFORMATION: /note= "Norvaline"

10

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 63

(D) OTHER INFORMATION: /note= "Norleucine"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 74

(D) OTHER INFORMATION: /note= "Norvaline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

20

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
1 5 10
His Leu Ile Xaa Cys His Ser Lys Lys Lys Cys Glu
15 20
Glu Xaa Ala Ala Lys Leu Val Ala Xaa Gly Ile Asn
25 30 35
Ala Val Ser Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
40 45
Ile Pro Thr Ala Gly Asp Val Val Val Ala Ser
50 55 60
Asp Ala Xaa Met Thr Gly Tyr Ser Gly Asp Phe Asp
65 70
Ser Xaa Ile Asp Cys Asn Thr Cys Val
75 80

30

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

35

This Sheet Has Been Replaced



(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 3

(D) OTHER INFORMATION: /note= "Norvaline"

5

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 7

(D) OTHER INFORMATION: /note=

"V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

10

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 16

(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 26

(D) OTHER INFORMATION: /note= "Norleucine"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 33

(D) OTHER INFORMATION: /note= "Norleucine"

20

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 46

(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 63

(D) OTHER INFORMATION: /note= "Norleucine"

25

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 74

(D) OTHER INFORMATION: /note= "Norvaline"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Lys Ala Xaa Pro Leu Glu Val Val Lys Gly Gly Arg
1 5 10

35

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His Leu Ile Xaa Cys His Ser Arg Lys Lys Cys Asp
 15 20
 Glu Xaa Ala Ala Lys Leu Val Ala Xaa Gly Ile Asn
 25 30 35
 Ala Val Ser Tyr Tyr Arg Gly Leu Asp Xaa Ser Val
 40 45
 5 Ile Pro Thr Ala Gly Asp Val Val Val Val Ala Ser
 50 55 60
 Asp Ala Xaa Met Thr Gly Tyr Ser Gly Asp Phe Asp
 65 70
 Ser Xaa Ile Asp Cys Asn Thr Cys Val
 75 80

10 (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"

20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note=
 "V0.30;A0.30;T0.10;F0.10;Y0.10;Norvaline0.10"

25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /note= "Norleucine"

30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 35

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(B) LOCATION: 46
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 63
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 74
(D) OTHER INFORMATION: /note= "Norvaline"

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Lys	Ala	Xaa	Pro	Leu	Glu	Val	Xaa	Lys	Gly	Gly	Arg
1				5					10		
His	Leu	Ile	Xaa	Cys	His	Ser	Arg	Lys	Lys	Cys	Asp
	15						20				
Glu	Xaa	Ala	Ala	Lys	Leu	Val	Ala	Xaa	Gly	Ile	Asn
25				30					35		
Ala	Val	Ser	Tyr	Tyr	Arg	Gly	Leu	Asp	Xaa	Ser	Val
		40					45				
Ile	Pro	Thr	Ala	Gly	Asp	Val	Val	Val	Val	Ala	Ser
	50				55					60	
Asp	Ala	Xaa	Met	Thr	Gly	Tyr	Ser	Gly	Asp	Phe	Asp
			65						70		
Ser	Xaa	Ile	Asp	Cys	Asn	Thr	Cys	Val			
20		75					80				

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys	Lys	Lys	Lys	Ala	Ile	Pro	Leu	Glu	Val	Ile	Lys
1				5					10		
Gly	Gly	Arg	His	Leu	Ile	Phe	Cys	His	Ser	Arg	Arg
	15						20				
Arg	Cys	Asp	Glu	Leu	Ala	Ala	Lys	Leu	Val	Ala	Leu
25				30					35		

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Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp
 40 45
 Val Ser Val Ile Pro Thr Ser Gly Asp Val Val Val
 50 55 60
 Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly
 65 70
 5 Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val
 75 80

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Lys Phe Pro Gly Gly Gly Gln Ile Met
 1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 62 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 9
 (D) OTHER INFORMATION: /note= "Ornithine"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 22
 (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site



(B) LOCATION: 44
(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 58
(D) OTHER INFORMATION: /note= "Hydroxyproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 64 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix). FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "P0.90;G0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= "Ornithine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= "T0.70;N0.30"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 24

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(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 33

(D) OTHER INFORMATION: /note= "Norvaline"

5

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 39

(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

10

(B) LOCATION: 46

(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 51

(D) OTHER INFORMATION: /note= "T0.90;P0.10"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 60

(D) OTHER INFORMATION: /note= "Hydroxyproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

20

Lys Lys Lys Ser Thr Ile Pro Lys Pro Gln Xaa Lys

1 5 10

Thr Lys Arg Asn Thr Asn Lys Arg Pro Gln Asp Xaa

15 20

Lys Phe Pro Gly Gly Gly Gln Ile Xaa Gly Gly Val

25 30 35

25 Tyr Leu Xaa Pro Arg Arg Gly Pro Arg Xaa Gly Leu

40 45

Arg Ala Thr Arg Lys Thr Thr Glu Arg Ser Gln Xaa

50 55 60

Arg Gly Arg Arg

(2) INFORMATION FOR SEQ ID NO:25:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 64 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

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- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7
 (D) OTHER INFORMATION: /note= "P0.90;G0.10"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 11
 (D) OTHER INFORMATION: /note= "Ornithine"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 13
 (D) OTHER INFORMATION: /note= "T0.70;N0.30"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 24
 (D) OTHER INFORMATION: /note= "Norvaline"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 27
 (D) OTHER INFORMATION: /note= "Hydroxyproline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /note= "Norvaline"
- 20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 39
 (D) OTHER INFORMATION: /note= "Norleucine"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 46
 (D) OTHER INFORMATION: /note= "Norleucine"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 51
 (D) OTHER INFORMATION: /note= "T0.90;P0.10"
- 30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 60
 (D) OTHER INFORMATION: /note= "Hydroxyproline"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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

10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 64 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "P0.90;G0.10"

20

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "Ornithine"

(ix) FEATURE:

25

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /note= "T0.70;N0.30"

(ix) FEATURE:

30

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 24
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 33
- (D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

35

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 39

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(D) OTHER INFORMATION: /note= "Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 46

(D) OTHER INFORMATION: /note= "Norleucine"

5

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 51

(D) OTHER INFORMATION: /note= "T0.90;P0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

10

(B) LOCATION: 53

(D) OTHER INFORMATION: /note= "Ornithine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 55

(D) OTHER INFORMATION: /note= "T0.25;A0.25;E0.25;K0.25"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 60

(D) OTHER INFORMATION: /note= "Hydroxyproline"

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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

30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ile Ile Pro Asp Arg Glu Val Leu Tyr Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= "Norvaline"

15

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 33
(D) OTHER INFORMATION: /note= "Norleucine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

20 Lys Lys Lys Ser Gly Lys Pro Thr Ile Ile Pro Asp
1 5 10
Arg Glu Xaa Leu Tyr Arg Glu Phe Asp Asp Met Glu
15 20
Asp Cys Ser Gln His Leu Pro Tyr Xaa Asp Gln Gly
25 30 35
Met Met Leu Ala Glu Asn Phe Lys Gln Lys Ala Val
40 45
25 Gly Leu
50

(2) INFORMATION FOR SEQ ID NO:29:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (ix) FEATURE:
(A) NAME/KEY: Modified-site

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(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "S0.80;N0.20"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note=
"G0.80;D0.10;Q0.10"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "K0.60;R0.40"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note=
"P0.80;V0.10;A0.10"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "I0.60;V0.40"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note=
"I0.60;V0.20;A0.20"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= "R0.80;K0.20"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note=
"R0.30;Q0.50;E0.20"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /note= "E0.80;A0.20"

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- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 27
 (D) OTHER INFORMATION: /note= "S0.40;A0.60"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 28
 (D) OTHER INFORMATION: /note= "Q0.40;S0.60"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 29
 (D) OTHER INFORMATION: /note= "H0.80;K0.10;R0.10"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 30
 (D) OTHER INFORMATION: /note= "L0.80;A0.20"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /note= "P0.80;A0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 32
20 (D) OTHER INFORMATION: /note= "Y0.80;L0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /note= "Norleucine"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 35
 (D) OTHER INFORMATION: /note= "Q0.80;E0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 37
30 (D) OTHER INFORMATION: /note= "M0.80;Q0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 38
 (D) OTHER INFORMATION: /note= "M0.40;Q0.40;R0.20"
- 35 (ix) FEATURE:

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(A) NAME/KEY: Modified-site
(B) LOCATION: 39
(D) OTHER INFORMATION: /note=
"L0.80;M0.10;I0.10"

(ix) FEATURE:

5 (A) NAME/KEY: Modified-site
(B) LOCATION: 43
(D) OTHER INFORMATION: /note= "F0.80;L0.20"

(ix) FEATURE:

10 (A) NAME/KEY: Modified-site
(B) LOCATION: 45
(D) OTHER INFORMATION: /note= "Q0.80;S0.20"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 47
(D) OTHER INFORMATION: /note= "A0.80;I0.20"

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Lys Lys Lys Ser Gly Lys Pro Thr Ile Ile Pro Asp.
1 5 10
Arg Glu Xaa Leu Tyr Arg Glu Phe Asp Asp Met Glu
15 20
Asp Cys Ser Gln His Leu Pro Tyr Xaa Asp Gln Gly
25 30 35
20 Met Met Leu Ala Glu Asn Phe Lys Gln Lys Ala Val
40 45
Gly Leu Val Lys Phe Pro Gly Gly Gly Asn Ile
50 55

(2) INFORMATION FOR SEQ ID NO:30:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "S0.80;N0.20"

(ix) FEATURE:

35 (A) NAME/KEY: Modified-site



(B) LOCATION: 5
(D) OTHER INFORMATION: /note=
"G0.80;D0.10;Q0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "K0.60;R0.40"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note=
"P0.80;V0.10;A0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "I0.60;V0.40"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note=
"I0.60;V0.20;A0.20"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= "R0.80;K0.20"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note=
"R0.30;Q0.50;E0.20"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /note= "E0.80;A0.20"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 27
(D) OTHER INFORMATION: /note= "S0.40;A0.60"

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- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 28
 (D) OTHER INFORMATION: /note= "Q0.40;S0.60"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 29
 (D) OTHER INFORMATION: /note=
 "H0.80;K0.10;R0.10"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 30
 (D) OTHER INFORMATION: /note= "L0.80;A0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /note= "P0.80;A0.20"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 32
 (D) OTHER INFORMATION: /note= "Y0.80;L0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /note= "Norvaline"
- 20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 35
 (D) OTHER INFORMATION: /note= "Q0.80;E0.20"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 37
 (D) OTHER INFORMATION: /note= "M0.80;Q0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 38
 (D) OTHER INFORMATION: /note=
 "M0.40;Q0.40;R0.20"
- 30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 39
 (D) OTHER INFORMATION: /note=
 "L0.80;M0.10;I0.10"
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(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 43
 (D) OTHER INFORMATION: /note= "F0.80;L0.20"

5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 45
 (D) OTHER INFORMATION: /note= "Q0.80;S0.20"

10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 47
 (D) OTHER INFORMATION: /note= "A0.80;I0.20"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 48
 (D) OTHER INFORMATION: /note= "Norvaline"

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Lys	Lys	Lys	Ser	Gly	Lys	Pro	Thr	Ile	Ile	Pro	Asp.
1				5					10		
Arg	Glu	Xaa	Leu	Tyr	Arg	Glu	Phe	Asp	Asp	Met	Glu
		15					20				
Asp	Cys	Ser	Gln	His	Leu	Pro	Tyr	Xaa	Asp	Gln	Gly
25					30					35	
20	Met	Met	Leu	Ala	Glu	Asn	Phe	Lys	Gln	Lys	Ala
			40					45			Xaa
Gly	Leu	Val	Lys	Phe	Pro	Gly	Gly	Gly	Asn	Ile	
	50					55					

(2) INFORMATION FOR SEQ ID NO:31:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 41 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 5
 (D) OTHER INFORMATION: /note= "P0.80;L0.10;V0.10"

35 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7



- (D) OTHER INFORMATION: /note= "Norvaline"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 9
 (D) OTHER INFORMATION: /note=
 "TO.50;S0.40;P0.10"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 12
 (D) OTHER INFORMATION: /note=
 "K0.40;D0.40;R0.20"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note=
 "E0.50;V0.40;Q0.10"
- 20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 18
 (D) OTHER INFORMATION: /note= "P0.90;A0.10"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 19
 (D) OTHER INFORMATION: /note= "V0.80;T0.20"
- 30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 20
 (D) OTHER INFORMATION: /note= "Norvaline"
- 35 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 21
 (D) OTHER INFORMATION: /note=
 "H0.80;L0.10;A0.10"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 24
 (D) OTHER INFORMATION: /note= "P0.80;A0.20"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 27
 (D) OTHER INFORMATION: /note= "P0.90;S0.10"
- (ix) FEATURE:

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(A) NAME/KEY: Modified-site
(B) LOCATION: 28
(D) OTHER INFORMATION: /note= "Hydroxyproline"

(ix) FEATURE:

5

(A) NAME/KEY: Modified-site
(B) LOCATION: 29
(D) OTHER INFORMATION: /note=
"K0.80;P0.10;R0.10"

(ix) FEATURE:

10

(A) NAME/KEY: Modified-site
(B) LOCATION: 30
(D) OTHER INFORMATION: /note=
"S0.40;A0.40;K0.10;Q0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 31
(D) OTHER INFORMATION: /note= "P0.80;T0.20"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 33
(D) OTHER INFORMATION: /note=
"V0.50;I0.40;T0.10"

(ix) FEATURE:

20

(A) NAME/KEY: Modified-site
(B) LOCATION: 34
(D) OTHER INFORMATION: /note= "Hydroxyproline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 38
(D) OTHER INFORMATION: /note= "K0.40;R0.60"

25

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 39
(D) OTHER INFORMATION: /note= "K0.70;R0.30"

(ix) FEATURE:

30

(A) NAME/KEY: Modified-site
(B) LOCATION: 41
(D) OTHER INFORMATION: /note= "T0.90;A0.10"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Asp Tyr Asn Pro Pro Leu Xaa Glu Thr Trp Lys Lys
1 5 10

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Pro Glu Tyr Glu Pro Pro Val Xaa His Gly Cys Pro
 15 20
 Leu Pro Pro Xaa Lys Ser Pro Pro Val Xaa Pro Pro
 25 30 35
 Arg Lys Lys Arg Thr
 40

5

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS: /
 (A) LENGTH: 42 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 10
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 6
 (D) OTHER INFORMATION: /note=
 "P0.80;L0.10;V0.10"
 15
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 8
 (D) OTHER INFORMATION: /note= "Norvaline"
 20
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 10
 (D) OTHER INFORMATION: /note=
 "TO.50;S0.40;P0.10"
 25
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 13
 (D) OTHER INFORMATION: /note=
 "K0.40;D0.40;R0.20"
 30
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 17
 (D) OTHER INFORMATION: /note=
 "E0.50;V0.40;Q0.10"
 35
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 19
 (D) OTHER INFORMATION: /note= "P0.90;A0.10"

35

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- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 20
 (D) OTHER INFORMATION: /note= "V0.80;T0.20"
- 5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 21
 (D) OTHER INFORMATION: /note= "Norvaline"
- 10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 22
 (D) OTHER INFORMATION: /note= "H0.80;L0.10;A0.10"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 25
 (D) OTHER INFORMATION: /note= "P0.80;A0.20"
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 28
 (D) OTHER INFORMATION: /note= "P0.90;S0.10"
- 20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 29
 (D) OTHER INFORMATION: /note= "Hydroxyproline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 30
 (D) OTHER INFORMATION: /note= "K0.80;P0.10;R0.10"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /note= "S0.40;A0.40;K0.10;Q0.10"
- 30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 32
 (D) OTHER INFORMATION: /note= "P0.80;T0.20"
- 35 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 34
 (D) OTHER INFORMATION: /note= "V0.50;I0.40;T0.10"

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(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 35
 (D) OTHER INFORMATION: /note= "Hydroxyproline"

5 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 39
 (D) OTHER INFORMATION: /note= "K0.40;R0.60"

10 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 40
 (D) OTHER INFORMATION: /note= "K0.70;R0.30"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 42
 (D) OTHER INFORMATION: /note= "T0.90;A0.10"

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Pro Asp Tyr Asn Pro Pro Leu Xaa Glu Thr Trp Lys
 1 5 10
Lys Pro Glu Tyr Glu Pro Pro Val Xaa His Gly Cys
 15 20
Pro Leu Pro Pro Xaa Lys Ser Pro Pro Val Xaa Pro
 25 30 35
20 Pro Arg Lys Lys Arg Thr
 40

(2) INFORMATION FOR SEQ ID NO:33:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 44 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 8
 (D) OTHER INFORMATION: /note=
 "P0.80;L0.10;V0.10"

35 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 10

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(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12

(D) OTHER INFORMATION: /note=
"TO.50;S0.40;P0.10"

5

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 15

(D) OTHER INFORMATION: /note=
"K0.40;D0.40;R0.20"

10

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 19

(D) OTHER INFORMATION: /note=
"E0.50;V0.40;Q0.10"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 21

(D) OTHER INFORMATION: /note= "P0.90;A0.10"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 22

(D) OTHER INFORMATION: /note= "V0.80;T0.20"

20

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 23

(D) OTHER INFORMATION: /note= "Norvaline"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 24

(D) OTHER INFORMATION: /note=
"H0.80;L0.10;A0.10"

25

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 27

(D) OTHER INFORMATION: /note= "P0.80;A0.20"

30

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 30

(D) OTHER INFORMATION: /note= "P0.90;S0.10"

35

(ix) FEATURE:

This Sheet Has Been Replaced



- (A) NAME/KEY: Modified-site
- (B) LOCATION: 31
- (D) OTHER INFORMATION: /note= "Hydroxyproline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 32
- (D) OTHER INFORMATION: /note= "K0.80;P0.10;R0.10"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 33
- (D) OTHER INFORMATION: /note= "S0.40;A0.40;K0.10;Q0.10"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 34
- (D) OTHER INFORMATION: /note= "P0.80;T0.20"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 36
- (D) OTHER INFORMATION: /note= "V0.50;I0.40;T0.10"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 37
- (D) OTHER INFORMATION: /note= "Hydroxyproline"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 41
- (D) OTHER INFORMATION: /note= "K0.40;R0.60"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 42
- (D) OTHER INFORMATION: /note= "K0.70;R0.30"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 44
- (D) OTHER INFORMATION: /note= "T0.90;A0.10"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Ornithine"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Xaa Pro Asp Tyr Asn Pro Pro Leu Xaa Glu Thr
 1 5 10
 Trp Lys Lys Pro Glu Tyr Glu Pro Pro Val Xaa His
 15 20
 5 Gly Cys Pro Leu Pro Pro Xaa Lys Ser Pro Pro Val
 25 30 35
 Xaa Pro Pro Arg Lys Lys Arg Thr
 40

(2) INFORMATION FOR SEQ ID NO:34:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 20 (B) LOCATION: 16
 (D) OTHER INFORMATION: /note= "Norvaline"
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 24
 (D) OTHER INFORMATION: /note= "Hydroxyproline"
- 25 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 32
 (D) OTHER INFORMATION: /note= "Hydroxyproline"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

30 Pro Leu Xaa Glu Thr Trp Lys Lys Pro Glu Tyr Glu
 1 5 10
 Pro Pro Val Xaa His Gly Cys Pro Leu Pro Pro Xaa
 15 20
 Lys Ser Pro Pro Val Pro Pro Xaa Arg Lys Lys Arg
 25 30 35
 35 Thr Lys

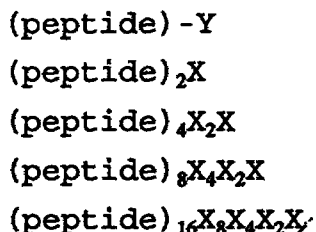
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WE CLAIM:

1. A peptide composition comprising at least one linear or one branched peptide represented by the formula



wherein Y is an OH or NH₂ group on the carboxyl group of the C terminal amino acid of (peptide), X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage, and

said peptide moiety, (peptide), is specifically immunoreactive with hepatitis C virus (HCV) antibodies, wherein said peptide moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5-34.

2. The peptide composition of Claim 1 comprising a mixture of two or more of said peptides.

3. The peptide composition of Claim 1, wherein one or more of said peptides is conjugated to a carrier.

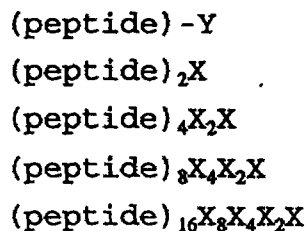
4. The peptide composition of Claim 1 wherein said peptide is linear.

5. The peptide composition of Claim 4 wherein said peptide is peptide 19, 25, 28, 29 or 33.

6. The peptide composition of Claim 1, wherein said peptide is branched.

7. The peptide composition of Claim 6, wherein said peptide is peptide 27.

8. A peptide composition comprising at least one linear or one branched peptide represented by the formula





wherein Y is an OH or NH₂ group on the carboxyl group of the C terminal amino acid of (peptide), X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage, and

5 said peptide moiety, (peptide), is specifically immunoreactive with hepatitis C virus (HCV) antibodies, wherein said peptide moiety comprises peptide which substantially retains the frame of the 81 amino acid prototype sequence of SEQ ID NO:4, which is immunoreactive
10 with HCV NS-3 antibodies and which consists of the following substituted or degenerate positions:

position 3: norvaline, position 7: norvaline or Val:Ala:Thr:Phe:Tyr:Nvl at 3:3:1:1:1:1 ratio,
position 8: valine or norvaline, position 16:
15 norvaline, position 20: arginine, position 26: norleucine, position 33: norleucine, position 39: serine, position 46: norvaline, position 52: alanine, position 60: serine, position 63: norleucine, position 68: serine, and position 74: norvaline.

20 9. A peptide composition comprising at least one linear or one branched peptide represented by the formula

(peptide)-Y
(peptide)₂X
(peptide)₄X₂X
25 (peptide)₈X₄X₂X
(peptide)₁₆X₈X₄X₂X

wherein Y is an OH or NH₂ group on the carboxyl group of the C terminal amino acid of (peptide), X is an amino acid or an amino acid analog having two amino groups and one
30 carboxyl group, each group capable of forming a peptide bond linkage, and

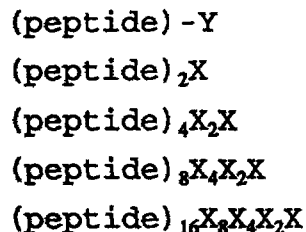
 said peptide moiety, (peptide), is specifically immunoreactive with hepatitis C virus (HCV) antibodies, wherein said peptide moiety comprises a peptide which
35 substantially retains the frame of the 61 amino acid prototype sequence of SEQ ID NO:3, which is immunoreactive



with HCV core antibodies and which consists of the following substituted or degenerate positions:

position 4: Pro:Gly at 9:1 ratio, position 8: ornithine, position 10: Thr:Asn at 7:3 ratio, position 16: lysine, position 21: norvaline, position 24: hydroxyproline, position 30: norvaline, position 36: valine or norleucine, position 43: norleucine, position 45: leucine, position 48: Thr:Pro at 9:1 ratio, position 52: threonine or Thr:Ala:Glu:Lys at 1:1:1:1 ratio, and position 57: hydroxyproline.

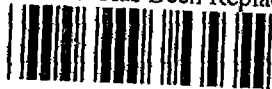
10. A peptide composition comprising at least one linear or one branched peptide represented by the formula



wherein Y is an OH or NH₂ group on the carboxyl group of the C terminal amino acid of (peptide), X is an amino acid or an amino acid analog having two amino groups and one carboxyl group, each group capable of forming a peptide bond linkage, and

said peptide moiety, (peptide), is specifically immunoreactive with hepatitis C virus (HCV) antibodies, wherein said peptide moiety comprises a peptide which substantially retains the frame of the 47 amino acid prototype sequence of SEQ ID NO:2 followed by the 9 amino acid prototype sequence of residues 21 to 29 of SEQ ID NO:3, which is immunoreactive with HCV NS-4 or core antibodies and which consists of the following substituted or degenerate positions:

position 1: Ser:Asn at 8:2 ratio, position 2: Gly:Asp:Gln at 8:1:1 ratio, position 3: Lys:Arg at 6:4 ratio, position 4: Pro:Val:Ala at 8:1:1 ratio, position 5: threonine, position 6: Ile:Val at 6:4 ratio, position 7: Ile:Val:Ala at 6:2:2 ratio, position 10: Arg:Lys at 8:2 ratio, position 12:



norvaline, position 15: Arg:Gln:Glu at 3:5:2 ratio,
 position 16: Glu:Ala at 8:2 ratio, position 19:
 aspartate, position 22: aspartate, position 24:
 Ser:Ala at 4:6 ratio, position 25: Gln:Ser at 4:6
 ratio, position 26: His:Lys:Arg at 8:1:1 ratio,
 position 27: Leu:Ala at 8:2 ratio, position 28:
 Pro:Ala at 8:2 ratio, position 29: Tyr:Leu at 8:2
 ratio, position 30: norleucine or norvaline, position
 31: aspartate, position 32: Gln:Glu at 8:2 ratio,
 position 34: Met:Gln at 8:2 ratio, position 35:
 Met:Gln:Arg at 4:4:2 ratio, position 36: Leu:Met:Ile
 at 8:1:1 ratio, position 39: asparagine, position 40:
 Phe:Leu at 8:2 ratio, position 42: Gln:Ser at 8:2
 ratio, position 44: Ala:Ile at 8:2 ratio, or position
 45: valine, and position 55: asparagine.

11. A peptide composition comprising at least one
 linear or one branched peptide represented by the formula

(peptide)-Y

(peptide)₂X

(peptide)₄X₂X

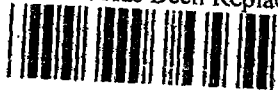
(peptide)₈X₄X₂X

(peptide)₁₆X₈X₄X₂X

wherein Y is an OH or NH₂ group on the carboxyl group of
 the C terminal amino acid of (peptide), X is an amino acid
 or an amino acid analog having two amino groups and one
 carboxyl group, each group capable of forming a peptide
 bond linkage, and said peptide moiety, (peptide), is
 specifically immunoreactive with hepatitis C virus (HCV)
 antibodies, wherein said peptide moiety comprises a
 peptide which substantially retains the frame of the 44
 amino acid prototype sequence of SEQ ID NO:1, which is
 immunoreactive with HCV NS-5 antibodies and which consists
 of the following substituted or degenerate positions:

position 2: ornithine, position 8: Pro:Leu:Val at
 8:1:1 ratio, position 10: norvaline, position 12:

Thr:Ser:Pro at 5:4:1 ratio, position 15: Lys:Asp:Arg
 at 4:4:2 ratio, position 17: glutamate, position 19:



5 Glu:Val:Gln at 5:4:1 ratio, position 21: Pro:Ala at
9:1 ratio, position 22: Val:Thr at 8:2 ratio,
position 23: norvaline, position 24: His:Leu:Ala at
8:1:1 ratio, position 27: Pro:Ala at 8:2 ratio,
position 30: Pro:Ser at 9:1 ratio, position 31:
10 hydroxyproline, position 32: Lys:Pro:Arg at 8:1:1
ratio, position 33: Ser:Ala:Lys: Gln at 4:4:1:1
ratio, position 34: Pro:Thr at 8:2 ratio, position
36: Val:Ile: Thr at 5:4:1 ratio, position 37:
hydroxyproline, position 41: Lys:Arg at 4:6 ratio,
15 position 42: Lys:Arg at 7:3 ratio, and position 44:
Thr:Ala at 9:1 ratio.

12. A peptide composition comprising Mixture A,
Mixture B or Mixture C.

15 13. The peptide composition of Claim 12 wherein said
composition comprises Mixture A.

14. A method of detecting HCV antibodies which
comprises using an effective amount of a peptide
composition according to any one of Claims 1 to 13 in an
immunoassay procedure.

20 15. A method of detecting HCV infection which
comprises contacting an effective amount of a peptide
composition of any one of Claims 1 to 13 with a body
fluid, tissue or tissue extract in an immunoassay
procedure for a time sufficient to form a complex between
25 said peptide composition and any antibody in said fluid,
said tissue, or said tissue extract, and subjecting said
complex to a detecting means.

30 16. The method of Claim 14 wherein said immunoassay
procedure is an ELISA assay, a sandwich assay or a PHA
assay.

17. The method of Claim 15 wherein said immunoassay
procedure is an ELISA assay, a sandwich assay or a PHA
assay.

35 18. A kit for detection of HCV antibodies or
diagnosis of HCV infection comprising a first container
adapted to contain the peptide composition of any one of



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Claims 1 to 13.

19. The kit of Claim 18 wherein said kit is an ELISA assay kit, a sandwich assay kit or PHA assay kit.

20. An ELISA test kit for detection of HCV antibodies or diagnosis of HCV infection comprising

(a) a container having a solid phase coated with the peptide composition of any one of Claims 1 to 13;

(b) a negative control sample;

(c) a positive control sample;

(d) specimen diluent; and

(e) antibodies to human IgG, said antibodies labeled with a reporter molecule.

21. A peptide of any one of Claims 1 to 13.

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