



(86) Date de dépôt PCT/PCT Filing Date: 2001/09/26

(87) Date publication PCT/PCT Publication Date: 2002/05/23

(45) Date de délivrance/Issue Date: 2012/04/03

(85) Entrée phase nationale/National Entry: 2003/04/01

(86) N° demande PCT/PCT Application No.: US 2001/030062

(87) N° publication PCT/PCT Publication No.: 2002/040997

(30) Priorités/Priorities: 2000/10/02 (US09/677,822);
2001/01/23 (US09/768,080)

(51) Cl.Int./Int.Cl. *G01N 33/549* (2006.01),
G01N 33/50 (2006.01), *G01N 33/68* (2006.01)

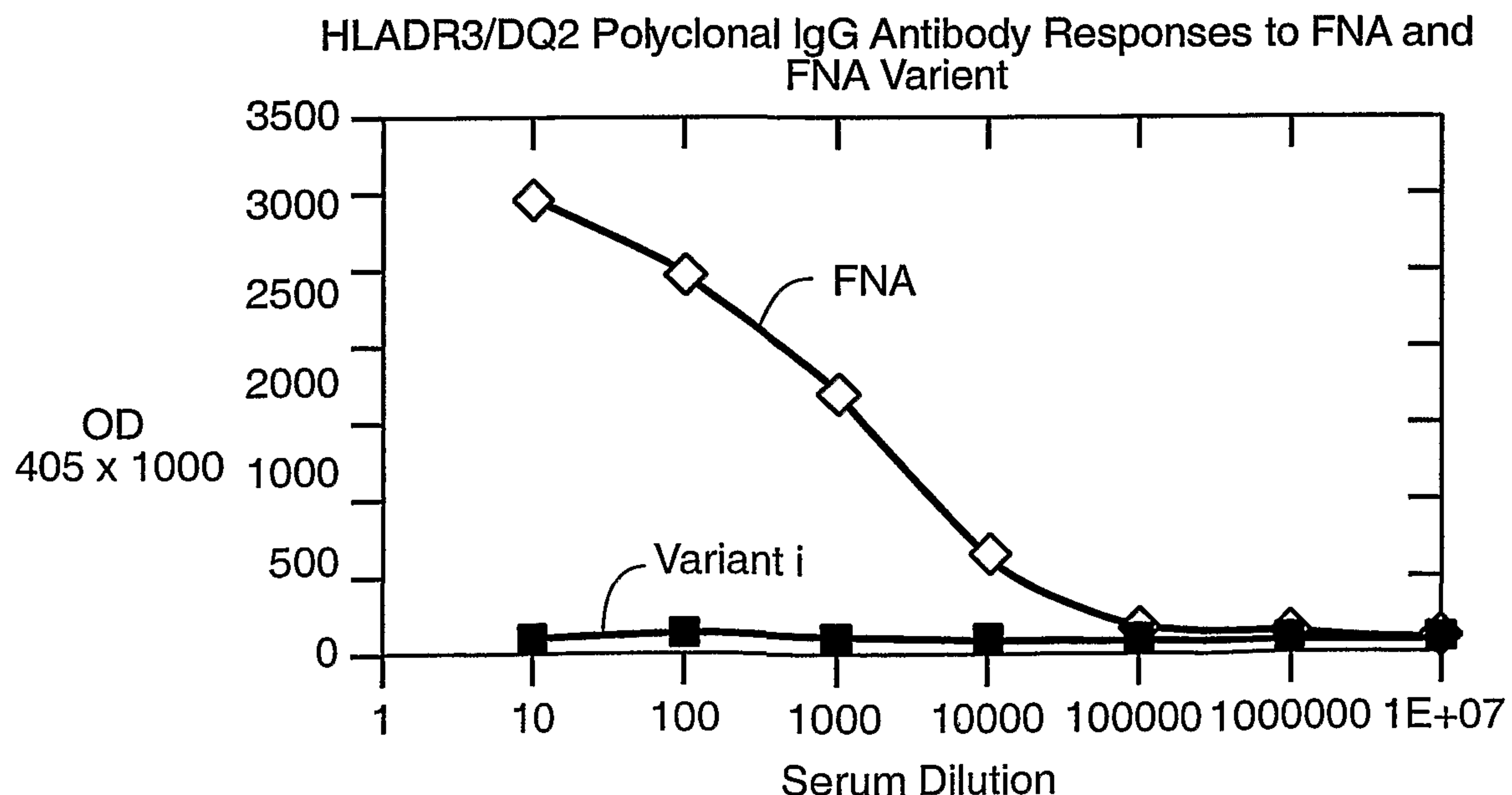
(72) Inventeurs/Inventors:
ESTELL, DAVID A., US;
HARDING, FIONA A., US

(73) Propriétaire/Owner:
GENENCOR INTERNATIONAL, INC., US

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PROTEINES PRODUISANT UNE REPONSE IMMUNOGENE MODIFIEE ET PROCEDES DE FABRICATION
ET D'UTILISATION DE CES PROTEINES

(54) Title: PROTEINS PRODUCING AN ALTERED IMMUNOGENIC RESPONSE AND METHODS OF MAKING AND
USING THE SAME



(57) Abrégé/Abstract:

The present invention relates to a novel methods and compositions for producing hyper and hypo allergenic compositions. Specifically, the present invention comprises neutralizing or reducing the ability of T-cells to recognize epitopes and thus prevent sensitization of an individual to the protein. Alternatively, T-cell epitopes are mutated to produce increased immunogenic reactions.

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

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 May 2002 (23.05.2002)

PCT

(10) International Publication Number
WO 02/040997 A2

(51) International Patent Classification⁷: **G01N 33/53**

(US). **HARDING, Fiona, A.** [US/US]; 772 Lewis Street, Santa Clara, CA 95050 (US).

(21) International Application Number: PCT/US01/30062

(74) **Agent: STONE, Christopher, L.**; GENENCOR INTERNATIONAL, INC., 925 Page Mill Road, Palo Alto, CA 94304 (US).

(22) International Filing Date:
26 September 2001 (26.09.2001)

(25) Filing Language: English

(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(26) Publication Language: English

(30) **Priority Data:**
09/677,822 2 October 2000 (02.10.2000) US
09/768,080 23 January 2001 (23.01.2001) US

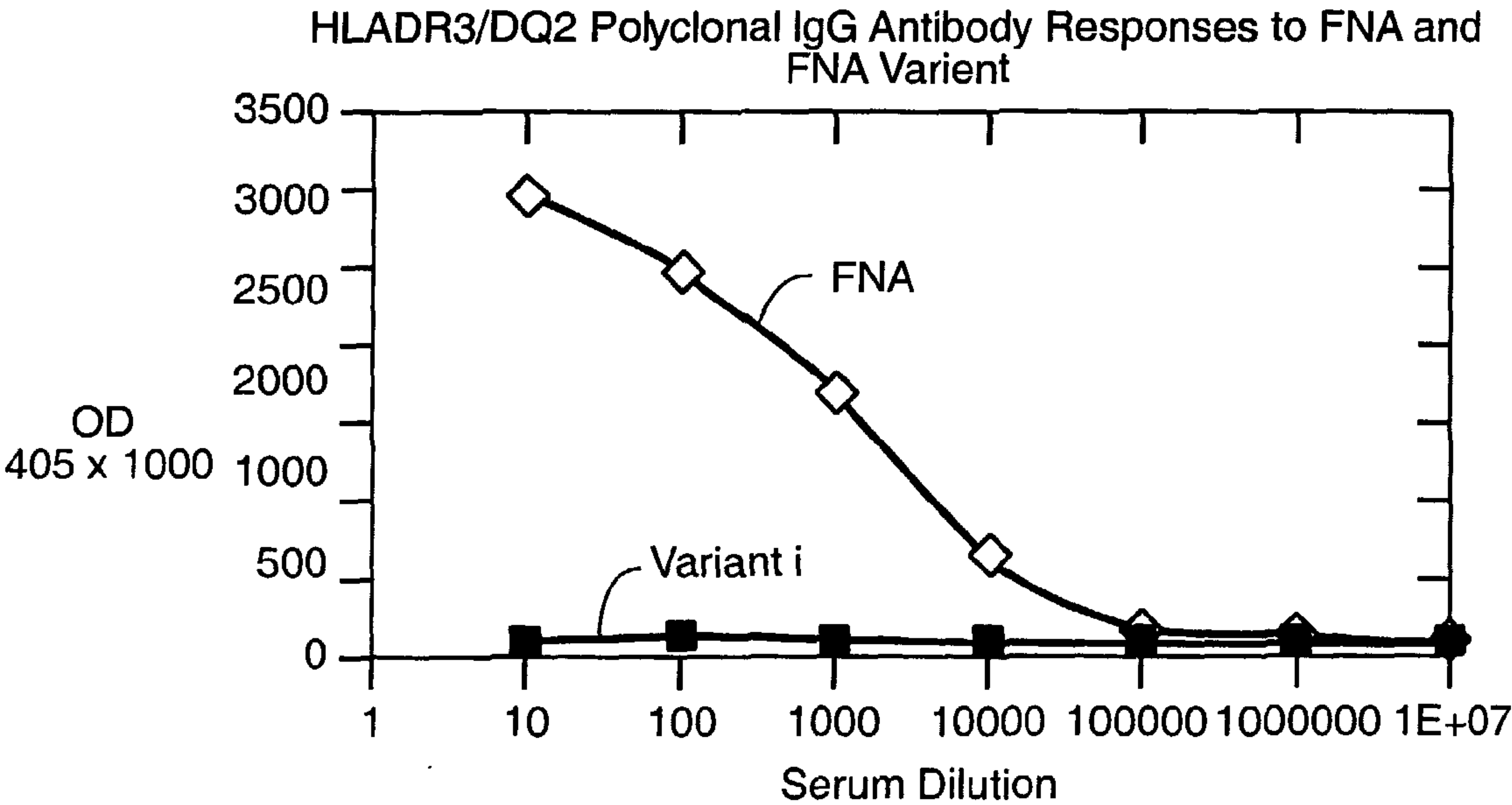
(71) **Applicant (for all designated States except US): GENENCOR INTERNATIONAL, INC.** [US/US]; 925 Page Mill Road, Palo Alto, CA 94304 (US).

(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) **Inventors; and**
(75) **Inventors/Applicants (for US only): ESTELL, David, A.** [UG/UG]; 248 Woodbridge Circle, San Mateo, CA 94403

[Continued on next page]

(54) **Title:** PROTEINS PRODUCING AN ALTERED IMMUNOGENIC RESPONSE AND METHODS OF MAKING AND USING THE SAME



(57) **Abstract:** The present invention relates to a novel methods and compositions for producing hyper and hypo allergenic compositions. Specifically, the present invention comprises neutralizing or reducing the ability of T-cells to recognize epitopes and thus prevent sensitization of an individual to the protein. Alternatively, T-cell epitopes are mutated to produce increased immunogenic reactions.

WO 02/040997 A2

WO 02/040997 A2



Published:

— *without international search report and to be republished
upon receipt of that report*

(15) Information about Correction:

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

(48) Date of publication of this corrected version:

25 July 2002

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

PROTEINS PRODUCING AN ALTERED IMMUNOGENIC RESPONSE AND METHODS OF MAKING AND USING THE SAME

5

BACKGROUND OF THE INVENTION

Proteins used in industrial, pharmaceutical and commercial applications are of increasing prevalence. As a result, the increased exposure due to this prevalence has been responsible for some safety hazards caused by the sensitization of certain persons to those peptides, whereupon
10 subsequent exposure causes extreme allergic reactions which can be injurious and even fatal. For example, proteases are known to cause dangerous hypersensitivity in some individuals. As a result, despite the usefulness of proteases in industry, e.g., in laundry detergents, cosmetics, textile treatment etc., and the extensive research performed in the field to provide improved proteases which have, for example, more effective stain removal under detergency conditions; the use of
15 proteases in industry has been problematic due to their ability to produce a hypersensitive allergenic response in some humans.

Much work has been done to alleviate these problems. Among the strategies explored to reduce immunogenic potential of protease use have been improved production processes which reduce potential contact by controlling and minimizing workplace concentrations of dust particles or
20 aerosol carrying airborne protease, improved granulation processes which reduce the amount of dust or aerosol actually produced from the protease product, and improved recovery processes to reduce the level of potentially allergenic contaminants in the final product. However, efforts to reduce the allergenicity of protease, per se, have been relatively unsuccessful. Alternatively, efforts have been made to mask epitopes in protease which are recognized by immunoglobulin E (IgE) in
25 hypersensitive individuals (PCT Publication No. WO 92/10755) or to enlarge or change the nature of the antigenic determinants by attaching polymers or peptides/proteins to the problematic protease.

When an adaptive immune response occurs in an exaggerated or inappropriate form, the individual experiencing the reaction is said to be hypersensitive. Hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately and sometimes cause
30 inflammatory reactions and tissue damage. They can be provoked by many antigens; and the cause of a hypersensitivity reaction will vary from one individual to the next. Hypersensitivity does not normally manifest itself upon first contact with the antigen, but usually appears upon subsequent contact. One form of hypersensitivity occurs when an IgE response is directed against innocuous environmental antigens, such as pollen, dust-mites or animal dander. The resulting release of
35 pharmacological mediators by IgE-sensitized mast cells produces an acute inflammatory reaction with symptoms such as asthma or rhinitis.

Nonetheless, a strategy comprising modifying the IgE sites will not generally be successful in preventing the cause of the initial sensitization reaction. Accordingly, such strategies, while perhaps neutralizing or reducing the severity of the subsequent hypersensitivity reaction, will not
40 reduce the number or persons actually sensitized. For example, when a person is known to be hypersensitive to a certain antigen, the general, and only safe, manner of dealing with such a

- 2 -

situation is to isolate the hypersensitive person from the antigen as completely as possible. Indeed, any other course of action would be dangerous to the health of the hypersensitive individual. Thus, while reducing the danger of a specific protein for a hypersensitive individual is important, for industrial purposes it would be far more valuable to render a protein incapable of initiating the hypersensitivity reaction in the first place.

T-lymphocytes (T-cells) are key players in the induction and regulation of immune responses and in the execution of immunological effector functions. Specific immunity against infectious agents and tumors is known to be dependent on these cells and they are believed to contribute to the healing of injuries. On the other hand, failure to control these responses can lead to auto aggression. In general, antigen is presented to T-cells in the form of antigen presenting cells which, through a variety of cell surface mechanisms, capture and display antigen or partial antigen in a manner suitable for antigen recognition by the T-cell. Upon recognition of a specific epitope by the receptors on the surface of the T-cells (T-cell receptors), the T-cells begin a series of complex interactions, including proliferation, which result in the production of antibody by B-cells. While T-cells and B-cells are both activated by antigenic epitopes which exist on a given protein or peptide, the actual epitopes recognized by these mononuclear cells are generally not identical. In fact, the epitope which activates a T-cell to initiate the creation of immunologic diversity is quite often not the same epitope which is later recognized by B-cells in the course of the immunologic response. Thus, with respect to hypersensitivity, while the specific antigenic interaction between the T-cell and the antigen is a critical element in the initiation of the immune response to antigenic exposure, the specifics of that interaction, i.e., the epitope recognized, is often not relevant to subsequent development of a full blown allergic reaction.

PCT Publication No. WO 96/40791 discloses a process for producing polyalkylene oxide-polypeptide conjugates with reduced allergenicity using polyalkylene oxide as a starting material.

PCT Publication No. WO 97/30148 discloses a polypeptide conjugate with reduced allergenicity which comprises one polymeric carrier molecule having two or more polypeptide molecules coupled covalently thereto.

PCT Publication No. WO 96/17929 discloses a process for producing polypeptides with reduced allergenicity comprising the step of conjugating from 1 to 30 polymolecules to a parent polypeptide.

PCT Publication No. WO 92/10755 discloses a method of producing protein variants evoking a reduced immunogenic response in animals. In this application, the proteins of interest, a series of proteases and variants thereof, were used to immunize rats. The sera from the rats was then used to measure the reactivity of the polyclonal antibodies already produced and present in the immunized sera to the protein of interest and variants thereof. From these results, it was possible to determine whether the antibodies in the preparation were comparatively more or less reactive with the protein and its variants, thus permitting an analysis of which changes in the protein are likely to neutralize or reduce the ability of the Ig to bind. From these tests on rats, the conclusion was arrived at that changing any of subtilisin 309 residues corresponding to 127, 128, 129, 130, 131,

- 3 -

151, 136, 151, 152, 153, 154, 161, 162, 163, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 186, 193, 194, 195, 196, 197, 247, 251, 261 will result in a change in the immunological potential.

PCT Publication No. WO 94/10191 discloses low allergenic proteins comprising oligomeric forms of the parent monomeric protein, wherein the oligomer has substantially retained its activity.

5 While some studies have provided methods of reducing the allergenicity of certain proteins and identification of epitopes which cause allergic reactions in some individuals, the assays used to identify these epitopes generally involve measurement of IgE and IgG antibody in blood sera previously exposed to the antigen. However, once an Ig reaction has been initiated, sensitization has already occurred. Accordingly, there is a need for a method of determining epitopes which
10 cause sensitization in the first place, as neutralization of these epitopes will result in significantly less possibility for sensitization to occur, thus reducing the possibility of initial sensitization. There is also a need to produce proteins which produce an enhanced immunogenic response, and a need to identify naturally occurring proteins which produce a low immunogenic response. This invention meets these and other needs.

15 SUMMARY OF THE INVENTION

The present invention provides proteins which produce immunogenic responses as desired, methods of identifying and making such proteins, and methods of using such proteins. For example, as will be become apparent from the detailed description below, the methods and compositions
20 provided herein are useful in forming hyper- and hypo-allergenic compositions. As used herein, hyper and hypo means the composition produces a greater or lesser immunogenic response, respectively, than the same composition without the proteins of the present invention. Such compositions may include cleaning compositions, textile treatments, contact lens cleaning solutions or products, peptide hydrolysis products, waste treatment products, cosmetic formulations including
25 for skin, hair and oral care, pharmaceuticals such as blood clot removal products, research products such as enzymes and therapeutics including vaccines.

In one aspect of the invention, a polypeptide of interest is selected and provided herein. The polypeptide of interest is preferably one having a T-cell epitope and is then varied as described below. However, polypeptides of interest may also be selected based on naturally occurring
30 properties and not altered. Moreover, polypeptides of interest may be selected which do not have a T-cell epitope, and altered so as to have a T-cell epitope.

In one aspect of the invention provided herein is a variant of a polypeptide of interest comprising a T-cell epitope. The variant differs from the polypeptide of interest by having an altered T-cell epitope such that said variant and said polypeptide produce different immunogenic responses
35 in an individual. The variant can be prepared and selected to produce either a greater or lesser immunogenic response than said polypeptide of interest.

The polypeptide of interest can be any polypeptide of interest. In one aspect, the polypeptide is selected from the group consisting of enzymes, hormones, factors, vaccines and cytokines. In one embodiment, the polypeptide of interest is not recognized by said individual as
40 endogenous to said individual, or not recognized as "self". As indicated herein, the polypeptide of

- 4 -

interest may be an enzyme. In one embodiment, the enzyme is selected from the group consisting of lipase, cellulase, endo-glucosidase H, protease, carbohydrase, reductase, oxidase, isomerase, transferase, kinase and phosphatase. In preferred embodiments, the polypeptide of interest and the variant of said polypeptide of interest each comprise at least some of the same activity. For
5 example, if a variant of a protease is provided, said variant will produce an altered immunogenic response, but will retain detectable, and preferably comparable, protease activity.

Wherein a variant of a polypeptide of interest is provided, the T-cell epitope may be altered in a number of ways including by amino acid substitutions, deletions, additions and combinations thereof. Preferably, the T-cell epitope is altered by having amino acid substitutions. In one
10 embodiment herein, the amino acid substitutions are made to corresponding amino acids of a homolog of the polypeptide of interest, wherein the homolog does not comprise the same T-cell epitope in the corresponding position as the polypeptide of interest. In one aspect, the terminal portion of the polypeptide of interest comprising at least one T-cell epitope is replaced with a corresponding terminal portion of the homolog of the polypeptide of interest, wherein the
15 replacement produces said different immunogenic response.

In another embodiment provided herein, the nucleic acids encoding the polypeptides producing the desired immunogenic response are provided herein. Moreover, the invention includes expression vectors and host cells comprising the nucleic acids provided herein. Moreover, once the polypeptides and variants thereof of the present invention are identified, substantially homologous
20 sequences of or those sequences which hybridize to the polypeptides and variants can be identified and are provided herein. Homologous is further defined below, and can refer to similarity or identity, with identity being preferred. Preferably, the homologous sequences are amino acid sequences or nucleic acids encoding peptides having the activity of the polypeptides and variants provided herein.

In yet another aspect of the invention is a method for determining the immunogenic
25 response produced by a protein. In one embodiment, the method comprises (a) obtaining from a single blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells; (b) promoting differentiation in said solution of dendritic cells; (c) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with said protein; and (d) measuring the proliferation of T-cells in said step (c).

30 The methods of determining immunogenic responses produced by proteins can also be used to identify comparative immunogenic responses of proteins. Therefore, in one aspect, the method of determining immunogenic responses of proteins further comprises comparing immunogenic responses of one or more proteins. The proteins can be homologs of each other, variants of the same protein, different types of the same protein, for example, different proteases, or
35 different peptides of the same protein.

The invention further provides a method of altering the immunogenicity of a polypeptide of interest comprising determining the immunogenicity of said polypeptide; identifying a T-cell epitope in a said polypeptide; and altering said T-cell epitope so as to alter the immunogenicity of said polypeptide. As described herein, said altering can be done by altering a single amino acid or

- 5 -

switching a portion of the polypeptide of interest with a corresponding portion of a homolog, wherein the switch produces an altered immunogenic response.

Other aspects of the invention will be understood by the skilled artisan by the following description.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A, B1, B2 and B3 illustrate the DNA (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequence for *Bacillus amyloliquefaciens subtilisin* (BPN') and a partial restriction map of this gene.

Fig. 2 illustrates the conserved amino acid residues among subtilisins from *Bacillus amyloliquefaciens* (SEQ ID NO: 3) and *Bacillus lentus* (wild-type) (SEQ ID NO: 4).

10

Figs. 3A and 3B illustrate an amino acid sequence alignment of subtilisin type proteases from *Bacillus amyloliquefaciens* (BPN'), *Bacillus subtilis*, *Bacillus licheniformis* (SEQ ID NOS: 5 & 6) and *Bacillus lentus*. The symbol * denotes the absence of specific amino acid residues as compared to subtilisin BPN'.

15

Fig. 4 illustrates the additive T-cell response of 16 peripheral mononuclear blood samples to peptides corresponding to the *Bacillus lentus* protease (GG36). Peptide E05 includes the region comprising residues corresponding to 170-173 in protease from *Bacillus amyloliquefaciens*.

20

Fig. 5 illustrates the additive T-cell response of 10 peripheral mononuclear blood samples to peptides corresponding to the human subtilisin molecule. Peptides F10, F9, F8 and F7 all contain the amino acid sequence DQMD corresponding to the region comprising residues corresponding to 170-173 in protease from *Bacillus amyloliquefaciens* in the sequence alignment of Fig. 3.

Fig. 6A and 6B/6C illustrate amino acid strings corresponding to peptides derived from the sequence of *Bacillus lentus* protease and a human subtilisin, respectively. (SEQ ID NOS: 7-207)

Fig. 7 illustrates the amino acid sequence of human subtilisin (SEQ ID NO: 208).

25

Fig. 8 illustrates an amino acid sequence alignment of BPN' (*Bacillus amyloliquefaciens*) protease, SAVINASE (*Bacillus lentus*) protease and human subtilisin (S2HSBT). (SEQ ID NO: 209)

Fig. 9 illustrates the T-cell response to peptides derived from *Bacillus lentus* protease in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease. Peptide E05 represents the region corresponding to 170-173 in protease from *Bacillus amyloliquefaciens*.

30

Fig. 10 illustrates the T-cell response to various alanine substitutions in the E05 *Bacillus lentus* protease peptide set in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease.

35

Fig. 11 illustrates the T-cell response to various alanine substitutions in the E05 protease peptide (an embodiment of the T-cell epitope designated unmodified sequence) set in a sample taken from an individual known to be hypersensitive to the protease; the sequences for each peptide are also shown. (SEQ ID NOS: 210-221)

Fig. 12 illustrates the percent responders to the human subtilisin molecule.

Fig. 13A illustrates the T-cell response of peptides derived from *Humicola insolens* endogluconase (Accession number A23635). Peptides A02 and F06 represent the region corresponding to residues 70-84 and 37-51, respectively, embodiments of the T-cell epitope, of

- 6 -

Humicola insolens endogluconase, wherein the full length sequence is shown in Fig. 13B and A02 and F06 are shown underlined and in bold. (SEQ ID NOS: 222-224)

Fig. 14A illustrates the T-cell response to peptides derived from *Thermomyces lanuginosa* lipase (Accession number AAC08588 and PID number g2997733). Peptides B02 and C06 represent the regions corresponding to residues 83-100 and 108-121, respectively, embodiments of the T-cell epitope, of *Thermomyces lanuginosa* lipase, wherein the full length sequence is shown in Fig. 14B and B02 and C06 are shown underlined and in bold. (SEQ ID NOS: 225-227)

Fig. 15A illustrates the T-cell response to peptides derived from *Streptomyces plicatus* endobeta-N-acetylglucosaminidase. (Accession number P04067). Peptide C06 represents the region corresponding to residues 126-140, an embodiment of the T-cell epitope, of *Streptomyces plicatus* endo-beta-N-acetylglucosaminidase, wherein the full length sequence is shown in Fig. 15B and C06 is shown underlined and in bold. (SEQ ID NOS: 228-229)

Fig. 16 illustrates the T-cell response to peptides derived from BPN'compiled for 22 individuals, wherein the sequences of preferred T-cell epitopes are indicated. (SEQ ID NOS: 230-231)

Fig. 17 illustrates the T-cell response to peptides derived from GG36 compiled for 22 individuals, wherein the sequences of embodiments of T-cell epitopes are indicated, GSISYPARYANAMAVGA and GAGLDIVAPGVNVQS being preferred. (SEQ ID NOS: 232-235)

Fig. 18 is an embodiment of a hybrid protein provided herein, where the N-terminus comprises N-terminal GG36 sequence and the C-terminus comprises C-terminal BPN'sequence, and wherein a comparison of the sequences with those shown in Fig. 8 indicates that the hybrid formed omits preferred T-cell epitopes of each protein. (SEQ ID NO: 236)

Figure 19 is a comparison of ELISA titers for *B. amyloliquefaciens* subtilisin and the ame subtilisin but engineered to contain a T-cell epitope from *B. lentis* subtilisin. Figure 19a represents the titer at 4 weeks; Figure 19b at 6 weeks, Figure 19c at 8 weeks and Figure 19d at 10 weeks.

Figure 20 is a time course study of ELISA titers for *B. amyloliquefaciens* subtilisin and the same subtilisin but engineered to contain a T-cell epitope from *B. lentis* subtilisin. Figure 20a represents the titer for a 1 µg dose of enzyme, Figure 20b a 5 µg dose and Figure 20c a 20 µg dose.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, a method for identifying T-cell epitopes is provided. Moreover, proteins including naturally occurring proteins which have relatively impotent or potent Tcell epitopes or no T-cell epitopes may be identified in accordance with the methods of the present invention. Thus, the present invention allows the identification and production of proteins which produce immunogenic responses as desired, including naturally occurring proteins as well as proteins which have been mutated to produce the appropriate response. It is understood that the terms protein, polypeptide and peptide are sometimes used herein interchangeably. Wherein a peptide is a portion of protein, the skilled artisan can understand this by the context in which the term is used.

In one embodiment, the present invention provides an assay which identifies epitopes and non-epitopes as follows: differentiated dendritic cells are combined with naive human CD4+ and/or

- 7 -

CD8+ T-cells and with a peptide of interest. More specifically, a method is provided wherein a T-cell epitope is recognized comprising the steps of: (a) obtaining from a single blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells; (b) promoting differentiation in said solution of dendritic cells; (c) combining said solution of differentiated dendritic cells and said naïve
5 CD4+ and/or CD8+ T-cells with a peptide of interest; (d) measuring the proliferation of T-cells in said step (c).

In one embodiment, the peptide of interest to be analyzed is derived from a polypeptide of interest. In the practice of the invention, it is possible to identify with precision the location of an epitope which can cause sensitization in an individual or sampling of individuals. In a preferred
10 embodiment of the invention, a series of peptide oligomers which correspond to all or part of the polypeptide of interest are prepared. For example, a peptide library is produced covering the relevant portion or all of the protein. In one embodiment, the manner of producing the peptides is to introduce overlap into the peptide library, for example, producing a first peptide corresponds to amino acid sequence 1-10 of the subject protein, a second peptide corresponds to amino acid
15 sequence 4-14 of the subject protein, a third peptide corresponds to amino acid sequence 7-17 of the subject protein, a fourth peptide corresponds to amino acid sequence 10-20 of the subject protein etc. until representative peptides corresponding to the entire molecule are created. By analyzing each of the peptides individually in the assay provided herein, it is possible to precisely identify the location of epitopes recognized by T-cells. In the example above, the greater reaction of
20 one specific peptide than its neighbors' will facilitate identification of the epitope anchor region to within three amino acids. After determining the location of these epitopes, it is possible to alter the amino acids within each epitope until the peptide produces a different T-cell response from that of the original protein. Alternatively, the epitope may be used in its original form to stimulate an immune response against a target, e.g. infectious agent or tumor cell. Moreover, proteins may be
25 identified herein which have desired high or low T-cell epitope potency which may be used in their naturally occurring forms.

"Antigen presenting cell" as used herein means a cell of the immune system which present antigen on their surface which is recognizable by receptors on the surface of T-cells. Examples of antigen presenting cells are dendritic cells, interdigitating cells, activated B-cells and macrophages.

30 "T-cell proliferation" as used herein means the number of T-cells produced during the incubation of T-cells with the antigen presenting cells, with or without antigen.

"Baseline T-cell proliferation" as used herein means T-cell proliferation which is normally seen in an individual in response to exposure to antigen presenting cells in the absence of peptide or protein antigen. For the purposes herein, the baseline T-cell proliferation level was determined on a
35 per sample basis for each individual as the proliferation of T-cells in response to antigen presenting cells in the absence of antigen.

"T-cell epitope" means a feature of a peptide or protein which is recognized by a T-cell receptor in the initiation of an immunologic response to the peptide comprising that antigen. Recognition of a T-cell epitope by a T-cell is generally believed to be via a mechanism wherein T-
40 cells recognize peptide fragments of antigens which are bound to class I or class II major

- 8 -

histocompatibility (MHC) molecules expressed on antigen-presenting cells (see e.g., Moeller, G. ed., "Antigenic Requirements for Activation of MHC-Restricted Responses," *Immunological Review*, Vol. 98, p. 187 (Copenhagen; Munksgaard) (1987)).

5 "Sample" as used herein comprises mononuclear cells which are naïve, *i.e.*, not sensitized, to the antigen in question.

"Homolog" as used herein means a protein or enzyme which has similar catalytic action, structure and/or use as the protein of interest. For purposes of this invention, a homolog and a protein of interest are not necessarily related evolutionarily, *e.g.*, same functional protein from different species. It is desirable to find a homolog that has a tertiary and/or primary structure similar to the protein of interest as replacement of the epitope in the protein of interest with an analogous segment from the homolog will reduce the disruptiveness of the change. Thus, closely homologous enzymes will provide the most desirable source of epitope substitutions. Alternatively, if possible, it is advantageous to look to human analogs for a given protein. For example, substituting a specific epitope in a bacterial subtilisin with a sequence from a human analog to subtilisin (*i.e.*, human subtilisin) should result in less allergenicity in the bacterial protein.

20 An "analogous" sequence may be determined by ensuring that the replacement amino acids show a similar function, the tertiary structure and/or conserved residues to the amino acids in the protein of interest at or near the epitope. Thus, where the epitope region contains, for example, an alpha-helix or a beta-sheet structure, the replacement amino acids should maintain that specific structure.

The epitopes determined according to the assay provided herein are then modified to reduce or augment the immunologic potential of the protein of interest. In a preferred embodiment, the epitope to be modified produces a level of T-cell proliferation of greater than three times the baseline T-cell proliferation in a sample. When modified, the epitope produces less than three times the baseline proliferation, preferably less than two times the baseline proliferation and most preferably less than or substantially equal to the baseline proliferation in a sample.

30 Preferably, the epitope is modified in one of the following ways: (a) the amino acid sequence of the epitope is substituted with an analogous sequence from a human homolog to the protein of interest; (b) the amino acid sequence of the epitope is substituted with an analogous sequence from a non-human homolog to the protein of interest, which analogous sequence produces a lesser immunogenic, *e.g.*, allergenic, response due to T-cell epitope recognition than that of the protein of interest; (c) the amino acid sequence of the epitope is substituted with a sequence which substantially mimics the major tertiary structure attributes of the epitope, but which produces a lesser immunogenic, *e.g.*, allergenic, response due to T-cell epitope recognition than that of the protein of interest; or (d) with any sequence which produces lesser immunogenic, *e.g.*, allergenic, response due to T-cell epitope recognition than that of the protein of interest.

40 However, one of skill will readily recognize that epitopes can be modified in other ways depending on the desired outcome. For example, if a T-cell vaccine is desired, it is contemplated the amino acid sequence of an epitope will be substituted with amino acids which increase the immunologic response to the peptide via enhanced MHC binding and/or T-cell recognition. In another

- 9 -

example, if altering an autoimmune response against self -antigens is desired, it is contemplated the amino acid sequence of an epitope will be substituted with amino acids that decrease or cause a shift in an inflammatory or other immune response.

The present invention extends to all proteins against which it is desired to modulate the immunogenic response, for example, peptides to be used as T-cell vaccines, or peptides or proteins to be used as therapeutic agents against, e.g., cancer, infectious diseases and autoimmune diseases. One of skill in the art will readily recognize the proteins and peptides of this invention are not necessarily native proteins and peptides. Indeed, in one embodiment of this invention, the assay described herein is used to determine the immunologic response of proteins from shuffled genes. For descriptions of gene shuffling and expression of such genes see, Stemmer, *Proc. Nat'l Acad. Sci. USA* 91:10747 (1994); Patten, et al., *Current Opinion in Biotechnol.* 8:724 (1997); Kuchner & Arnold, *Trends Biotechnol.* 15:523 (1997); Moore, et al., *J. Mol. Biol.* 272:336 (1997); Zhao, et al., *Nature Biotechnol.* 16:258 (1998); Giver, et al., *Proc. Nat'l Acad. Sci. USA* 95:12809 (1998); Harayama, *Trends Biotechnol.* 16:76 (1998); Lin, et al., *Biotechnol., Prog.* 15:467 (1999); and Sun, *J. Comput. Biol.* 6:77 (1999). The assay is used to predict the immunologic response of proteins encoded by shuffled genes. Once determined, the protein can be altered to modulate the immunologic response to that protein.

In addition to the above proteins and peptides, the present invention can be used to reduce the allergenicity of proteins. These proteins include, but are not limited to, glucanases, lipases, cellulases, endo-glucosidase Hs (endo-H), proteases, carbohydrases, reductases, oxidases, isomerases, transferases, kinases, phosphatases, amylases, etc. In addition to reducing the allergenicity to an animal, such as a human, of naturally occurring amino acid sequences, this invention encompasses reducing the allergenicity of a mutated human protein, e.g., a protein that has been altered to change the functional activity of the protein. In many instances, the mutation of human proteins to e.g., increase activity, results in the incorporation of new T-cell epitope in the mutated protein. The assay of this invention can be used to determine the presence of the new T-cell epitope and determine substitute amino acids that will reduce the allergenicity of the mutated protein.

Although this invention encompasses the above proteins and many others, for the sake of simplicity, the following will describe a particularly preferred embodiment of the invention, the modification of protease. Proteases are carbonyl hydrolases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "protease" means a naturally-occurring protease or a recombinant protease. Naturally-occurring proteases include α -aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallo-carboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid proteases are included, as well as endo and exo-proteases.

In one embodiment herein, hybrid polypeptides are provided. "Hybrid polypeptides" are proteins engineered from at least two different proteins, which are preferably homologs of one another. For example, a preferred hybrid polypeptide might have the N-terminus of a protein and the C-terminus of a homolog of the protein. In a preferred embodiment, the two terminal ends can be

- 10 -

combined to correspond to the full-length active protein. In a preferred embodiment, the homologs share substantial similarity but do not have identical T-cell epitopes. Therefore, in one embodiment, for example, a polypeptide of interest having one or more T-cell epitopes in the C-terminus may have the C-terminus replaced with the C-terminus of a homolog having a less potent T-cell epitope in the C-terminus, less T-cell epitopes, or no T-cell epitope in the C-terminus. Thus, the skilled artisan understands that by being able to identify T-cell epitopes among homologs, a variety of variants producing different immunogenic responses can be formed. Moreover, it is understood that internal portions, and more than one homolog can be used to produce the variants of the present invention.

More generally, the variants provided herein can be derived from the precursor amino acid sequence by the substitution, deletion, insertion, or combination thereof of one or more amino acids of the precursor amino acid sequence. Such modification is preferably of the "precursor DNA sequence" which encodes the amino acid sequence of the precursor enzyme, but can be by the manipulation of the precursor protein. Suitable methods for such manipulation of the precursor DNA sequence include methods disclosed herein, as well as methods known to those skilled in the art (see, for example, EP 0 328299, WO89/06279 and the US patents and applications already referenced herein).

Subtilisins are bacterial or fungal proteases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "subtilisin" means a naturally-occurring subtilisin or a recombinant subtilisin. A series of naturally-occurring subtilisins is known to be produced and often secreted by various microbial species. Amino acid sequences of the members of this series are not entirely homologous. However, the subtilisins in this series exhibit the same or similar type of proteolytic activity. This class of serine proteases shares a common amino acid sequence defining a catalytic triad which distinguishes them from the chymotrypsin related class of serine proteases. The subtilisins and chymotrypsin related serine proteases both have a catalytic triad comprising aspartate, histidine and serine. In the subtilisin related proteases the relative order of these amino acids, reading from the amino to carboxy terminus, is aspartate-histidine-serine. In the chymotrypsin related proteases, the relative order, however, is histidine-aspartate-serine. Thus, subtilisin herein refers to a serine protease having the catalytic triad of subtilisin related proteases. Examples include but are not limited to the subtilisins identified in Fig. 3 herein. Generally and for purposes of the present invention, numbering of the amino acids in proteases corresponds to the numbers assigned to the mature *Bacillus amyloliquefaciens* subtilisin sequence presented in Fig. 1.

"Recombinant", "recombinant subtilisin" or "recombinant protease" refer to a subtilisin or protease in which the DNA sequence encoding the subtilisin or protease is modified to produce a variant (or mutant) DNA sequence which encodes the substitution, deletion or insertion of one or more amino acids in the naturally-occurring amino acid sequence. Suitable methods to produce such modification, and which may be combined with those disclosed herein, include those disclosed in US Patent 4,760,025 (RE 34,606), US Patent 5,204,015 and US Patent 5,185,258.

"Non-human subtilisins" and the DNA encoding them may be obtained from many procaryotic and eucaryotic organisms. Suitable examples of procaryotic organisms include gram

- 11 -

negative organisms such as *E. coli* or *Pseudomonas* and gram positive bacteria such as *Micrococcus* or *Bacillus*. Examples of eucaryotic organisms from which subtilisin and their genes may be obtained include yeast such as *Saccharomyces cerevisiae*, fungi such as *Aspergillus* sp.

“Human subtilisin” means proteins of human origin which have subtilisin type catalytic activity, e.g., the kexin family of human derived proteases. An example of such a protein is represented by the sequence in Fig. 7. Additionally, derivatives or homologs of proteins provided herein, including those from non-human sources such as mouse or rabbit, which retain the essential activity of the peptide, such as the ability to hydrolyze peptide bonds, etc., have at least 50%, preferably at least 65% and most preferably at least 80%, more preferably at least 90%, and sometimes as much as 95 or 98% homology to the polypeptide of interest. In one embodiment, the polypeptide of interest is shown in the Figures.

The amino acid position numbers used herein refer to those assigned to the mature *Bacillus amyloliquefaciens* subtilisin sequence presented in Fig. 1. The invention, however, is not limited to the mutation of this particular subtilisin but extends to precursor proteases containing amino acid residues at positions which are “equivalent” to the particular identified residues in *Bacillus amyloliquefaciens* subtilisin. In a preferred embodiment of the present invention, the precursor protease is *Bacillus lentus* subtilisin and the substitutions, deletions or insertions are made at the equivalent amino acid residue in *B. lentus* corresponding to those listed above.

A residue (amino acid) of a precursor protease is equivalent to a residue of *Bacillus amyloliquefaciens* subtilisin if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in *Bacillus amyloliquefaciens* subtilisin (i.e., having the same or similar functional capacity to combine, react, or interact chemically). “Corresponding” as used herein generally refers to an analogous position along the peptide.

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus amyloliquefaciens* subtilisin primary sequence and particularly to a set of residues known to be invariant in subtilisins for which the sequence is known. For example, Fig. 2 herein shows the conserved residues as between *B. amyloliquefaciens* subtilisin and *B. lentus* subtilisin. After aligning the conserved residues, allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of *Bacillus amyloliquefaciens* subtilisin are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Conservation of the catalytic triad, Asp32/His64/Ser221 should be maintained.

For example, the amino acid sequence of subtilisin from *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus licheniformis* (carlsbergensis) and *Bacillus lentus* can be aligned to provide the maximum amount of homology between amino acid sequences. A comparison of these sequences shows that there are a number of conserved residues contained in each sequence. The conserved residues as between BPN' and *B. lentus* are identified in Fig. 2.

- 12 -

These conserved residues, thus, may be used to define the corresponding equivalent amino acid residues of *Bacillus amyloliquefaciens* subtilisin in other subtilisins such as subtilisin from *Bacillus lentus* (PCT Publication No. W089/06279 published July 13, 1989), the preferred protease precursor enzyme herein, or the subtilisin referred to as PB92 (EP 0 328 299), which is highly homologous to the preferred *Bacillus lentus* subtilisin. The amino acid sequences of certain of these subtilisins are aligned in Figs. 3A and 3B with the sequence of *Bacillus amyloliquefaciens* subtilisin to produce the maximum homology of conserved residues. As can be seen, there are a number of deletions in the sequence of *Bacillus lentus* as compared to *Bacillus amyloliquefaciens* subtilisin. Thus, for example, the equivalent amino acid for Val165 in *Bacillus amyloliquefaciens* subtilisin in the other subtilisins is isoleucine for *B. lentus* and *B. licheniformis*.

Thus, for example, the amino acid at position +170 is lysine (K) in both *B. amyloliquefaciens* and *B. licheniformis* subtilisins and arginine (R) in Savinase. In one embodiment of the protease variants of the invention, however, the amino acid equivalent to +170 in *Bacillus amyloliquefaciens* subtilisin is substituted with aspartic acid (D). The abbreviations and one letter codes for all amino acids in the present invention conform to the PatentIn User Manual (GenBank, Mountain View, CA) 1990, p.101.

Homologous sequences can also be determined by using a "sequence comparison algorithm." Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection.

An example of an algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul, *et al.*, *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. These initial neighborhood word hits act as starting points to find longer HSPs containing them. The word hits are expanded in both directions along each of the two sequences being compared for as far as the cumulative alignment score can be increased. Extension of the word hits is stopped when: the cumulative alignment score falls off by the quantity X from a maximum achieved value; the cumulative score goes to zero or below; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M'-5, N'-4, and a comparison of both strands.

- 13 -

The BLAST algorithm then performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, an amino acid sequence is considered similar to a protein such as a protease if the smallest sum probability in a comparison of the test amino acid sequence to a protein such as a protease amino acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

"Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor protein whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the precursor protein such as the protease and *Bacillus amyloliquefaciens* subtilisin (N on N, CA on CA, C on C and O on O) are within 0.13nm and preferably 0.1nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the protein such as the protease in question to the *Bacillus amyloliquefaciens* subtilisin. The best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available.

Equivalent residues which are functionally analogous to a specific residue of *Bacillus amyloliquefaciens* subtilisin are defined as those amino acids of the precursor protein such as a protease which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the *Bacillus amyloliquefaciens* subtilisin. Further, they are those residues of the precursor protein, for example, protease (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two of the side chain atoms of the residue lie within 0.13nm of the corresponding side chain atoms of *Bacillus amyloliquefaciens* subtilisin. The coordinates of the three dimensional structure of *Bacillus amyloliquefaciens* subtilisin are set forth in EPO Publication No. 0 251 446 (equivalent to US Patent 5,182,204) and can be used as outlined above to determine equivalent residues on the level of tertiary structure.

Some of the residues identified for substitution, insertion or deletion are conserved residues whereas others are not. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in a naturally-occurring sequence. The variants of the present invention include the mature forms of protein variants, as well as the pro- and prepro- forms of such protein variants. The prepro- forms are the preferred construction since this facilitates the expression, secretion and maturation of the protein variants.

- 14 -

"Prosequence" refers to a sequence of amino acids bound to the N-terminal portion of the mature form of a protein which when removed results in the appearance of the "mature" form of the protein. Many proteolytic enzymes are found in nature as translational proenzyme products and, in the absence of post-translational processing, are expressed in this fashion. A preferred
5 prosequence for producing protein variants such as protease variants is the putative prosequence of *Bacillus amyloliquefaciens* subtilisin, although other prosequences may be used.

A "signal sequence" or "presequence" refers to any sequence of amino acids bound to the N-terminal portion of a protein or to the N-terminal portion of a proprotein which may participate in the secretion of the mature or pro forms of the protein. This definition of signal sequence is a
10 functional one, meant to include all those amino acid sequences encoded by the N-terminal portion of the protein gene which participate in the effectuation of the secretion of protein under native conditions. The present invention utilizes such sequences to effect the secretion of the protein variants as defined herein. One possible signal sequence comprises the first seven amino acid residues of the signal sequence from *Bacillus subtilis* subtilisin fused to the remainder of the signal
15 sequence of the subtilisin from *Bacillus lentus* (ATCC 21536).

A "prepro" form of a protein variant consists of the mature form of the protein having a prosequence operably linked to the amino terminus of the protein and a "pre" or "signal" sequence operably linked to the amino terminus of the prosequence.

"Expression vector" refers to a DNA construct containing a DNA sequence which is operably
20 linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable
25 host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably as the plasmid is the most commonly used form of vector at present. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which are, or become, known in the art.

The "host cells" used in the present invention generally are procaryotic or eucaryotic hosts
30 which preferably have been manipulated by the methods disclosed in US Patent 4,760,025 (RE 34,606) to render them incapable of secreting enzymatically active endoprotease. A preferred host cell for expressing protein is the *Bacillus* strain BG2036 which is deficient in enzymatically active neutral protein and alkaline protease (subtilisin). The construction of strain BG2036 is described in
35 detail in US Patent 5,264,366. Other host cells for expressing protein include *Bacillus subtilis* 168 (also described in US Patent 4,760,025 (RE 34,606) and US Patent 5,264,366), as well as any suitable *Bacillus* strain such as *B. licheniformis*, *B. tentus*, etc.

Host cells are transformed or transfected with vectors constructed using recombinant DNA
40 techniques. These techniques can be found in any molecular biology practice guide, for example,

- 15 -

Sambrook *et al.* Molecular Cloning - A Laboratory Manual (2nd ed.) Vol. 1-3, Cold Springs Harbor Publishing (1989) ("Sambrook"); and Current Protocols in Molecular Biology, Ausubel *et al.* (eds.), Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1997 Supplement) ("Ausubel"). Such transformed host cells are capable of either
5 replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or prepro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

"Operably linked", when describing the relationship between two DNA regions, simply means that they are functionally related to each other. For example, a presequence is operably
10 linked to a peptide if it functions as a signal sequence, participating in the secretion of the mature form of the protein most probably involving cleavage of the signal sequence. A promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

The genes encoding the naturally-occurring precursor protein may be obtained in accord
15 with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protein of interest, preparing genomic libraries from organisms expressing the protein, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.

"Hybridization" is used to analyze whether a given DNA fragment or gene corresponds to a
20 DNA sequence described herein and thus falls within the scope of the present invention. Samples to be hybridized are electrophoresed through an agarose gel (for example, 0.8% agarose) so that separation of DNA fragments can be visualized by size. DNA fragments are typically visualized by ethidium bromide staining. The gel may be briefly rinsed in distilled H₂O and subsequently
25 depurinated in an appropriate solution (such as, for example, 0.25M HCl) with gentle shaking followed by denaturation for 30 minutes (in, for example, 0.4 M NaOH) with gentle shaking. A renaturation step may be included, in which the gel is placed in 1.5 M NaCl, 1M Tris, pH 7.0 with gentle shaking for 30 minutes.

The DNA should then be transferred onto an appropriate positively charged membrane, for
30 example, Maximum Strength Nytran Plus membrane (Schleicher & Schuell, Keene, N.H.), using a transfer solution (such as, for example, 6XSSC (900 mM NaCl, 90 mM trisodium citrate)). Once the transfer is complete, generally after about 2 hours, the membrane is rinsed in e.g., 2X SSC (2X SSC = 300 mM NaCl, 30 mM trisodium citrate) and air dried at room temperature. The membrane should then be prehybridized (for approximately 2 hours or more) in a suitable prehybridization solution
35 (such as, for example, an aqueous solution containing per 100 mL: 20-50 mL formamide, 25 mL of 20X SSPE (1X SSPE = 0.18 M NaCl, 1 mM EDTA, 10 mM NaH₂PO₄, pH 7.7), 2.5 mL of 20% SDS, and 1 mL of 10 mg/mL sheared herring or salmon sperm DNA). As would be known to one of skill in the art, the amount of formamide in the prehybridization solution may be varied depending on the nature of the reaction obtained according to routine methods. Thus, a lower amount of formamide
40 may result in more complete hybridization in terms of identifying hybridizing molecules than the

- 16 -

same procedure using a larger amount of formamide. On the other hand, a strong hybridization band may be more easily visually identified by using more formamide.

A DNA probe that is complementary or is nearly complementary to the DNA sequence of interest and is generally between 100 and 1000 bases in length is labeled (using, for example, the
5 Megaprime labeling system according to the instructions of the manufacturer) to incorporate ^{32}P in the DNA. The labeled probe is denatured by heating to 95°C for 5 minutes and immediately added to the membrane and prehybridization solution. The hybridization reaction should proceed for an appropriate time and under appropriate conditions, for example, for 18 hours at 37°C with gentle shaking or rotating. The membrane is rinsed (for example, in 2X SSC/0.3% SDS) and then washed
10 in an appropriate wash solution with gentle agitation. The stringency desired will be a reflection of the conditions under which the membrane (filter) is washed.

Specifically, the stringency of a given reaction (*i.e.*, the degree of homology necessary for successful hybridization) will depend on the washing conditions to which the filter is subjected after hybridization. "Low-stringency" conditions as defined herein will comprise washing a filter with a
15 solution of 0.2X SSC/0.1% SDS at 20°C for 15 minutes. "High-stringency" conditions comprise a further washing step comprising washing the filter a second time with a solution of 0.2X SSC/0.1% SDS at 37°C for 30 minutes.

After washing, the membrane is dried and the bound probe detected. If ^{32}P or another radioisotope is used as the labeling agent, the bound probe can be detected by autoradiography.
20 Other techniques for the visualization of other probes are well-known to those of skill. The detection of a bound probe indicates a nucleic acid sequence has the desired homology and is encompassed within this invention.

The cloned protein is then used to transform a host cell in order to express the protein. The protein gene is then ligated into a high copy number plasmid. This plasmid replicates in hosts in the
25 sense that it contains the well-known elements necessary for plasmid replication: a promoter operably linked to the gene in question (which may be supplied as the gene's own homologous promoter if it is recognized, *i.e.*, transcribed, by the host), a transcription termination and polyadenylation region (necessary for stability of the mRNA transcribed by the host from the protein gene in certain eucaryotic host cells) which is exogenous or is supplied by the endogenous
30 terminator region of the protein gene and, desirably, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antibiotic-containing media. High copy number plasmids also contain an origin of replication for the host, thereby enabling large numbers of plasmids to be generated in the cytoplasm without chromosomal limitations. However, it is within the scope herein to integrate
35 multiple copies of the protein gene into host genome. This is facilitated by procaryotic and eucaryotic organisms which are particularly susceptible to homologous recombination.

In one embodiment, the gene can be a natural gene such as that from *B. lentus* or *B. amyloliquefaciens*. Alternatively, a synthetic gene encoding a naturally-occurring or mutant precursor protein may be produced. In such an approach, the DNA and/or amino acid sequence of
40 the precursor protein is determined. Multiple, overlapping synthetic single-stranded DNA fragments

- 17 -

are thereafter synthesized, which upon hybridization and ligation produce a synthetic DNA encoding the precursor protein. An example of synthetic gene construction is set forth in Example 3 of US Patent 5,204,015.

Once the naturally-occurring or synthetic precursor protein gene has been cloned, a number of modifications are undertaken to enhance the use of the gene beyond synthesis of the naturally-occurring precursor protein. Such modifications include the production of recombinant proteins as disclosed in US Patent 4,760,025 (RE 34,606) and EPO Publication No. 0 251 446 and the production of protein variants described herein.

The following cassette mutagenesis method may be used to facilitate the construction of the protein variants of the present invention, although other methods may be used. First, the naturally-occurring gene encoding the protein is obtained and sequenced in whole or in part. Then the sequence is scanned for a point at which it is desired to make a mutation (deletion, insertion or substitution) of one or more amino acids in the encoded enzyme. The sequences flanking this point are evaluated for the presence of restriction sites for replacing a short segment of the gene with an oligonucleotide pool which when expressed will encode various mutants. Such restriction sites are preferably unique sites within the protein gene so as to facilitate the replacement of the gene segment. However, any convenient restriction site which is not overly redundant in the protein gene may be used, provided the gene fragments generated by restriction digestion can be reassembled in proper sequence. If restriction sites are not present at locations within a convenient distance from the selected point (from 10 to 15 nucleotides), such sites are generated by substituting nucleotides in the gene in such a fashion that neither the reading frame nor the amino acids encoded are changed in the final construction. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by M13 primer extension in accord with generally known methods. The task of locating suitable flanking regions and evaluating the needed changes to arrive at two convenient restriction site sequences is made routine by the redundancy of the genetic code, a restriction enzyme map of the gene and the large number of different restriction enzymes. Note that if a convenient flanking restriction site is available, the above method need be used only in connection with the flanking region which does not contain a site.

Once the naturally-occurring DNA or synthetic DNA is cloned, the restriction sites flanking the positions to be mutated are digested with the cognate restriction enzymes and a plurality of end termini-complementary oligonucleotide cassettes are ligated into the gene. The mutagenesis is simplified by this method because all of the oligonucleotides can be synthesized so as to have the same restriction sites, and no synthetic linkers are necessary to create the restriction sites.

In one aspect of the invention, the objective is to secure a variant protein having altered allergenic potential as compared to the precursor protein, since decreasing such potential enables safer use of the enzyme. While the instant invention is useful to lower allergenic potential, the mutations specified herein may be utilized in combination with mutations known in the art to result altered thermal stability and/or altered substrate specificity, modified activity or altered alkaline stability as compared to the precursor.

- 18 -

Accordingly, the present invention is directed to altering the capability of the T-cell epitope which includes residue positions 170-173 in *Bacillus lentus* to induce T-cell proliferation. One particularly preferred embodiment of the invention comprises making modification to either one or all of R170D, Y171Q and/or N173D. Similarly, as discussed in detail above, it is believed that the modification of the corresponding residues in any protein will result in a the neutralization of a key T-cell epitope in that protein. Thus, in combination with the presently disclosed mutations in the region corresponding to amino acid residues 170-173, substitutions at positions corresponding to N76D/S103A/V104I/G159D optionally in combination with one or more substitutions selected from the group consisting of positions corresponding to V68A, T213R, A232V, Q236H, Q245R, and T260A of *Bacillus amyloliquefaciens* subtilisin may be used, in addition to decreasing the allergenic potential of the variant protein of the invention, to modulate overall stability and/or proteolytic activity of the enzyme. Similarly, the substitutions provided herein may be combined with mutation at the Asparagine (N) in *Bacillus lentus* subtilisin at equivalent position +76 to Aspartate (D) in combination with the mutations S103A/V104I/G159D and optionally in combination with one or more substitutions selected from the group consisting of positions corresponding to V68A, T213R, A232V, Q236H, Q245R, and T260A of *Bacillus amyloliquefaciens* subtilisin, to produce enhanced stability and/or enhanced activity of the resulting mutant enzyme.

The most preferred embodiments of the invention include the following specific combinations of substituted residues corresponding to positions:

N76D/S103A/V104I/G159D/K170D/Y171Q/S173D;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D /Q236H;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D /Q236H/Q245R;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/A232V/Q236H/Q245R; and

V68A/N76D//S103A/V104I/G159D/K170D/Y171Q/S173D/T213R/A232V/Q236H/

Q245R/T260A of *Bacillus amyloliquefaciens* subtilisin. These substitutions are preferably made in *Bacillus lentus* (recombinant or native-type) subtilisin, although the substitutions may be made in any *Bacillus* protein.

Based on the screening results obtained with the variant proteins, the noted mutations noted above in *Bacillus amyloliquefaciens* subtilisin are important to the proteolytic activity, performance and/or stability of these enzymes and the cleaning or wash performance of such variant enzymes.

Many of the protein variants of the invention are useful in formulating various detergent compositions. A number of known compounds are suitable surfactants useful in compositions comprising the protein mutants of the invention. These include nonionic, anionic, cationic, anionic or zwitterionic detergents, as disclosed in US 4,404,128 to Barry J. Anderson and US 4,261,868 to Jiri Flora, et al. A suitable detergent formulation is that described in Example 7 of US Patent 5,204,015. The art is familiar with the different formulations which can be used as leaning compositions. In addition to typical cleaning compositions, it is readily understood that the protein variants of the present invention may be used for any purpose that native or wild-type proteins are used. Thus, these variants can be used, for example, in bar or liquid soap applications, dishcare formulations, contact lens cleaning solutions or products, peptide hydrolysis,

- 19 -

waste treatment, textile applications, as fusion-cleavage enzymes in protein production, etc. The variants of the present invention may comprise, in addition to decreased allergenicity, enhanced performance in a detergent composition (as compared to the precursor). As used herein, enhanced performance in a detergent is defined as increasing cleaning of certain enzyme sensitive stains such as grass or blood, as determined by usual evaluation after a standard wash cycle.

Proteins, particularly proteases of the invention can be formulated into known powdered and liquid detergents having pH between 6.5 and 12.0 at levels of about .01 to about 5% (preferably .1% to .5%) by weight. These detergent cleaning compositions can also include other enzymes such as known proteases, amylases, cellulases, lipases or endoglycosidases, as well as builders and stabilizers.

The addition of proteins, particularly proteases of the invention to conventional cleaning compositions does not create any special use limitation. In other words, any temperature and pH suitable for the detergent is also suitable for the present compositions as long as the pH is within the above range, and the temperature is below the described protein's denaturing temperature. In addition, proteins of the invention can be used in a cleaning composition without detergents, again either alone or in combination with builders and stabilizers.

The variant proteins of the present invention can be included in animal feed such as part of animal feed additives as described in, for example, US 5,612,055; US 5,314,692; and US 5,147,642.

One aspect of the invention is a composition for the treatment of a textile that includes variant proteins of the present invention. The composition can be used to treat for example silk or wool as described in publications such as RD 216,034; EP 134,267; US 4,533,359; and EP 344,259.

The variants can be screened for proteolytic activity according to methods well known in the art. Preferred protease variants include multiple substitutions at positions corresponding to:

N76D/S103A/V104I/G159D/K170D/Y171Q/S173D;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/Q236H;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/Q236H/Q245R;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/A232V/Q236H/Q245R; and

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/T213R/A232V/Q236H/Q245R/T260A of *Bacillus amyloliquefaciens* subtilisin.

The proteins of this invention exhibit modified immunogenicity when compared to their precursor proteins. In preferred embodiments, the proteins exhibit reduced allergenicity. In other embodiments, the proteins exhibit increased immunogenicity. The increase in immunogenicity is manifested by an increase in B-cell or humoral immunological response, by an increase in T-cell or cellular immunological response, or by an increase in both B and T cell immunological responses.

One of skill will readily recognize that the uses of the proteins of this invention will be determined, in large part, on the immunological properties of the proteins. For example, enzymes that exhibit reduced allergenicity can be used in cleaning compositions. "Cleaning compositions" are compositions that can be used to remove undesired compounds from substrates, such as fabric, dishes, contact lenses, other solid substrates, hair (shampoos), skin (soaps and creams), etc.

- 20 -

Proteins, in particular, cellulases, proteases, and amylases, with reduced allergenicity can also be used in the treatment of textiles. "Textile treatment" comprises a process wherein textiles, individual yarns or fibers that can be woven, felted or knitted into textiles or garments are treated to effect a desired characteristic. Examples of such desired characteristics are "stone-washing", depilling, dehairing, desizing, softening, and other textile treatments well known to those of skill in the art.

Therapeutic proteins against which individuals mount an immune response are also included in the invention. In particular, individuals who lack endogenous production of the protein are susceptible to forming neutralizing antibodies and become refractile to treatment. Likewise, modifications of a protein may introduce new epitopes that are potentially immunogenic. Methods of the invention can be used to identify and modify epitopes in , e.g., human Factor VIII, to prevent neutralizing responses.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.

The following is presented by way of example and is not to be construed as a limitation to the scope of the claims.

EXAMPLES

Example 1

Assay for the Identification of Peptide T-Cell Epitopes Using Naïve Human T-Cells

Fresh human peripheral blood cells were collected from "naïve" humans, i.e., persons not known to be exposed to or sensitized to *Bacillus lentus* protease, for determination of antigenic epitopes in protease from *Bacillus lentus* and human subtilisin. Naïve humans is intended to mean that the individual is not known to have been exposed to or developed a reaction to protease in the past. Peripheral mononuclear blood cells (stored at room temperature, no older than 24 hours) were prepared for use as follows: Approximately 30 mls of a solution of buffy coat preparation from one unit of whole blood was brought to 50 ml with Dulbecco's phosphate buffered solution (DPBS) and split into two tubes. The samples were underlaid with 12.5 ml of room temperature lymphoprep density separation media (Nycomed density 1.077 g/ml). The tubes were centrifuged for thirty minutes at 600G. The interface of the two phases was collected, pooled and washed in DPBS. The

- 21 -

cell density of the resultant solution was measured by hemocytometer. Viability was measured by trypan blue exclusion.

From the resulting solution, a differentiated dendritic cell culture was prepared from the peripheral blood mononuclear cell sample having a density of 108 cells per 75 ml culture flask in a solution as follows:

(1) 50 ml of serum free AIM V media (Gibco) was supplemented with a 1:100 dilution beta-mercaptoethanol (Gibco). The flasks were laid flat for two hours at 37°C in 5% CO2 to allow adherence of monocytes to the flask wall.

(2) Differentiation of the monocyte cells to dendritic cells was as follows: nonadherent cells were removed and the resultant adherent cells (monocytes) combined with 30 ml of AIM V, 800 units/ml of GM-CSF (Endogen) and 500 units/ml of IL-4 (Endogen); the resulting mixture was cultured for 5 days under conditions at 37°C in 5% CO2. After five days, the cytokine TNFa (Endogen) was added to 0.2 units/ml, and the cytokine IL-1a (Endogen) was added to a final concentration of 50 units/ml and the mixture incubated at 37°C in 5% CO2 for two more days.

(3) On the seventh day, Mitomycin C was added to a concentration of 50 microgram/ml was added to stop growth of the now differentiated dendritic cell culture. The solution was incubated for 60 minutes at 37°C in 5% CO2. Dendritic cells were collected by gently scraping the adherent cells off the bottom of the flask with a cell scraper. Adherent and non-adherent cells were then centrifuged at 600G for 5 minutes, washed in DPBS and counted.

(4) The prepared dendritic cells were placed into a 96 well round bottom array at 2x10⁴/well in 100 microliter total volume of AIM V media.

CD4+ T cells were prepared from frozen aliquots of the peripheral blood cell samples used to prepare the dendritic cells using the human CD4+ Collect Kit (Biotex) as per the manufacturers instructions with the following modifications: the aliquots were thawed and washed such that approximately 108 cells will be applied per Collect column; the cells were resuspended in 4 ml DPBS and 1 ml of the Cell reagent from the Collect Kit, the solution maintained at room temperature for 20 minutes. The resultant solution was centrifuged for five minutes at 600G at room temperature and the pellet resuspended in 2 ml of DPBS and applied to the Collect columns. The effluent from the columns was collected in 2% human serum in DPBS. The resultant CD4+ cell solution was centrifuged, resuspended in AIMV media and the density counted.

The CD4+ T-cell suspension was resuspended to a count of 2x10⁶/ml in AIM V media to facilitate efficient manipulation of the 96 well plate.

Peptide antigen is prepared from a 1M stock solution in DMSO by dilution in AIM V media at a 1:10 ratio. 10 microliters of the stock solution is placed in each well of the 96 well plate containing the differentiated dendritic cells. 100 microliter of the diluted CD4+ T-cell solution as prepared

- 22 -

above is further added to each well. Useful controls include diluted DMSO blanks, and tetanus toxoid positive controls.

The final concentrations in each well, at 210 microliter total volume are as follows:

2x10⁴ CD4+

5 2x10⁵ dendritic cells (R:S of 10:1)

5 mM peptide

Example 2

Identification of T-Cell Epitopes in Protease 10 from *Bacillus lentus* and Human subtilisin

Peptides for use in the assay described in Example 1 were prepared based on the *Bacillus lentus* and human subtilisin amino acid sequence. Peptide antigens were designed as follows. From the full length amino acid sequence of either human subtilisin or *Bacillus lentus* protease provided in Figure 1, 15mers were synthetically prepared, each 15mer overlapping with the previous
15 and the subsequent 15mer except for three residues.

Peptides used correspond to amino acid residue strings in *Bacillus lentus* as provided in Figure 8, and peptides correspond to amino acid residues in human subtilisin as provided in Figure 7. The peptides used corresponding to the proteases is provided in Fig. 6. All tests were performed at least in duplicate. All tests reported displayed robust positive control responses to the antigen
20 tetanus toxoid. Responses were averaged within each experiment, then normalized to the baseline response. A positive event was recorded if the response was at least 3 times the baseline response.

The immunogenic response (*i.e.*, T-cell proliferation) to the prepared peptides from human subtilisin and *Bacillus lentus* was tallied and is provided in Figures 4 and 5, respectively. T-cell proliferation was measured by the incorporated tritium method. The results shown in Figures 4 and
25 5 as a comparison of the immunogenic additive response in 10 individuals (Figure 4) and 16 individuals (Figure 5) to the various peptides. Response is indicated as the added response wherein 1.0 equals a baseline response for each sample. Thus, in Figure 4, a reading of 10.0 or less is the baseline response and in Figure 5 a reading of 16.0 or less the baseline response. The greater the response, the more potent the T-cell epitope is considered.

30 As indicated in Figures 4 and 5, the immunogenic response of the naïve blood samples from unsensitized individuals showed a marked allergenic response at the peptide fragment from *Bacillus lentus* corresponding to residues 170-173 of *Bacillus amyloliquefaciens* protease. As expected, the corresponding fragment in human subtilisin evokes merely baseline response.

Fig. 9 shows the T-cell response to peptides derived from *Bacillus lentus* protease in a
35 sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease. Peptide E05 represents the region corresponding to 170-173 in protease from *Bacillus amyloliquefaciens*. As shown in Fig. 9, the hypersensitive individual was highly responsive to the T-cell epitope represented by the peptide E05. This result confirms that, by practicing the assay according to the invention, it is possible to predict the major epitopes identified by the T-cells of a hypersensitive
40 individual.

- 23 -

Fig. 10 shows the T-cell response to various alanine substitutions in the E05 peptide derived from *Bacillus lentus* protease in a sample taken from an individual known to be hypersensitive to *Bacillus lentus* protease. Alanine substitutions were used as substitutions for the purpose of determining the role of any specific residue within the epitope. The legend of Figure 10 refers to the position of the peptide in which an alanine was substituted, i.e., in peptide E06 (sequence GSISYPARYANAMAV), G to A = 2, S to A = 3, I to A = 4, S to A = 5, Y to A = 6, P to A = 7, R to A = 8, Y to A = 9, N to A = 10, M to A = 11 and V to A = 12. As indicated in Figure 10, substitution of either of the residues R170A, Y171A and/or N173A in protease from *Bacillus lentus* results in dramatically reduced response in the hypersensitive individual's blood sample.

From these results, it is apparent that the residues 170, 171 and 173 are largely responsible for the initiation of allergic reaction within the protease from *Bacillus lentus*.

Example 3

Identification of T-Cell Epitopes in Cellulase from *Humicola insolens* (Carezyme®)

The procedure described above was performed on peptides derived from a cellulase from *Humicola insolens* (Carezyme ® from Novo Nordisk). As can be seen from Figure 13, 2 T-cell epitopes were discovered, A01 and F06.

Example 4

Identification of T-Cell Epitopes in Lipase from *Thermomyces Lanuginosa* (Lipolase®)

The procedure described in Example 2 was performed on peptides derived from a lipase from *Thermomyces lanuginosa* (Lipolase ® from Novo Nordisk). As can be seen from Figure 14, two T-cell epitopes were discovered, A12 and C06. Peptide E03 effected slightly increased T-cell proliferation in the naïve donors, however, this peptide is consecutive to A12 and they represent one epitope. In this regard, the skilled artisan understands that the length of the epitopes can be varied, and the precise potency of the epitope, naturally occurring or mutated can be determined by the methods herein.

Example 5

Identification of T-Cell Epitopes in Endoglucanase H from *Streptomyces plicatus*

The procedure described in Example 2 was performed on peptides derived from endoglucanase H from *Streptomyces plicatus*. As can be seen from Figure 15, a single T-cell epitope was discovered, C06.

Example 6

Identification of T-Cell Epitopes in a Protease Hybrid (GG36-BPN')

After determining the location of a T-cell epitope, a protease hybrid was constructed using established protein engineering techniques. The hybrid was constructed so that a highly allergenic amino acid sequence of the protein was replaced with a corresponding sequence from a less

- 24 -

allergenic homolog. In this instance, the first 122 amino acids of the protease were derived from GG36, and the remaining amino acid sequence was derived from BPN'.

The hybrid was first tested from a 100 ppm sample in North American condition in 24 well assay at .5 ppm, superfixed swatches, liquid (Tide KT) at .5 in 24 well assay with 3K swatches, and in the N'N'-dimethyl Casein Assay, 5 g/l DMC in NA detergent, TNBS detection method.

The results are shown in Figures 16, 17 and 18.

Example 7

Identification of a Naturally Occuring Low Immunogenic Protein

Using the methods herein, proteinase K was identified as producing a lower immunogenic response than other commercially available proteases. Proteinase K as identified herein is from *Tritirachium Album limber*. For a general description of proteases and methodologies, see, Mathew, C.G.P. Isolation of high molecular weight eukaryotic DNA, in *Methods in Molecular Biology*, vol. 2: Nucleic Acids (Walker, J.M.,ed.), Humana, Clifton, NJ, (1984) pp. 31-34.

Example 8:

T-cell Epitope Introduced into a Non-allergenic Protein

It has been found that *Bacillus amyloliquefaciens* subtilisin is comparatively non-immunogenic when tested in Hartley strain guinea pigs. A related protein from *Bacillus lentis* is highly immunogenic. We had previously defined functional T cell epitopes in the *B. lentis* molecule which were not found in the *B. amyloliquefaciens* molecule, even though the sequences of interest were highly homologous. In order to test the principle that the presence of a functional T cell epitope can control the relative levels of antibody production, we created a *B. lentis*-like T cell epitope in the *B. amyloliquefaciens* molecule. This change was accomplished by the substitution of a single amino acid in the *B. amyloliquefaciens* sequence. *B. amyloliquefaciens* subtilisin and the T cell epitope modified variant of *B. amyloliquefaciens* subtilisin were tested in a guinea pig model of immunogenicity.

B. lentis and *B. amyloliquefaciens* subtilisin T cell epitope mapping: Guinea pigs were immunized with 20 µg/immunization of subtilisin from either *B. lentis* or *B. amyloliquefaciens*. Animals were immunized subcutaneously in adjuvant every two weeks for 10 to 12 weeks. A single cell suspension of guinea pig splenocytes was created from each animal's spleen. Cells were plated at 5×10^5 splenocytes per well in round bottom 96 well plates. 15-mer peptides off-set by 3 amino acids were synthesized by Mimotopes. Peptides were resuspended to 1 mM in DMSO. Peptides were added to the cells at a final concentration of 5 µM. Cultures were incubated for 5 days at 37 °, 5% CO₂. Wells were pulsed with 0.5 µCi tritiated thymidine, and allowed to incubate for an additional 18 hours. Wells were harvested, and thymidine incorporation assessed.

Two T cell epitopes were found in *B. lentis* subtilisin, and none were found in *B. amyloliquefaciens* subtilisin (>10 animals tested for these epitopes). The *B. lentis* T cell epitopes were found to comprise the following sequences:

- 25 -

IAALNNSIGVLGVAP (SEQ ID NO:237) and LEWAGNNGMHVANLSLGS (SEQ ID NO:238)

For SEQ ID NO:237, the similar sequence in *B. amyloliquefaciens* subtilisin is VAALNNSIGVLGVAP (SEQ ID NO:239). The similar region in *B. amyloliquefaciens* subtilisin for SEQ ID NO:238 was the much less homologous: IEWAIANNMDVINMSLG (SEQ ID NO:240).

5 SEQ ID NO:237 and the homologous region in the *B. amyloliquefaciens* subtilisin molecule (SEQ ID NO: 239) differ by one amino acid: In *B. lentis* subtilisin the first amino acid is an I, while it is a V in *B. amyloliquefaciens*. Therefore, we reasoned that if we changed the V in the *B. amyloliquefaciens* sequence to an I, we would create the *B. lentis* T cell epitope in the *B. amyloliquefaciens* backbone.

10 This molecule was created by standard molecular biological techniques, and was called *B. amyloliquefaciens* V72I. It was also known as GP002.

Guinea pig immunizations: Adult female Hartley guinea pigs were immunized with various doses of *B. amyloliquefaciens* subtilisin and GP002. The doses were 1, 5, 10, and 20 µg/dose. There were four animals for each dose. Animals were immunized subcutaneously with enzyme in
15 Complete Freund's Adjuvant for the first immunization. All subsequent

immunizations were performed in Incomplete Freund's adjuvant. Animals were immunized, and a serum sample taken, every two weeks.

ELISA: A direct ELISA was performed. Costart EIA plates were coated with 10 µg/ml of the
20 immunizing enzyme in PBS overnight at 4 °C. Plates were washed and blocked with 1% BSA in PBS. Serum samples were diluted in 1% BSA/PBS, and incubated on the enzymes coated plates for 1 hour. Serum samples were washed out, and biotinylated anti-guinea pig IgG was added at a 1:10,000 dilution in 1% BSA/PBS. The secondary reagent was incubated for 1 hour. The wells were washed, and avidin conjugated horse radish peroxidase was added to the wells at a 1:1000 dilution
25 in 1% BSA/PBS. After 30 minutes, the substrate (ABTS) was added and the OD₄₀₅ was read after 30 minutes.

Calculation of titers: Background was subtracted from the OD readings, and the results plotted for each individual guinea pig. A linear regression analysis was performed on the linear portion of the curve. The titer value was calculated from the linear regression equation for an OD =
30 0.5. These individual titers were then averaged.

Two guinea pigs in the 10 µg dose of GP001 died at 2 weeks into the study. The data for the 10 µg dose was therefore thrown out.

Two results are immediately apparent: first, the GP002 variant increased the titers of antigen-specific antibody over the entire time course for the lower doses of enzymes; and the GP002
35 variant increased titers of antigen-specific antibody for all doses of enzymes in the earliest time points.

At the extended time points and for the higher doses, the difference between *B. amyloliquefaciens* subtilisin and its variant were no longer apparent. See Figures 19 and 20.

From the Figures it is apparent that a single change in the amino acid sequence of *B. amyloliquefaciens* subtilisin significantly altered its immunogenicity.
40

- 26 -

Example 9Reduction of Allergenicity *in Vivo*

5 Given the ability to identify of human T cell epitopes, it is possible to modify their amino acid sequence to reduce activation of T cells and the subsequent immune response to the protein. However, to evaluate the *in vivo* effect of these changes, it is necessary to use an animal model that represents the ability of human HLA molecules to present the epitopes. For example, human T cell epitopes have been identified in the molecule BPN' in the regions 70-84 and 109-122 (see USSN

10 09/500,135, filed February 8, 2000; Figure 16).

 Substitutions in the amino acid sequence of these motifs led to reduced T cell proliferation *in vitro* using human cells representing a broad range of human HLA haplotypes. *In vitro* binding assays using EBV-transformed B cell lines demonstrated the peptides 70-84 and 109-123 bound to HLA DQ2 molecules. The substitutions that were found to reduce T-cell proliferation were

15 introduced into the coding sequence for FNA (BPN' with a Y217L substitution) for production of reduced immunogenic FNA variants.

 Transgenic mice expressing human HLA genes have been used to study epitopes presented to the immune system *in vivo*. Although the responding immune cells are of mouse origin, there is a strong correlation between the epitopes recognized in humans and mice. However, a

20 novel use of HLA transgenic mice is in the testing of variant proteins for reduced allergenic potential as a prediction of how human individuals will respond.

 To demonstrate this effect, both FNA and the FNA variant containing amino acid changes in the epitopes 70-84 and 109-123 were used to immunize HLA DR3/DQ2 transgenic mice that had been backcrossed onto I-Ab knockout mice (lacking the expression of endogenous I-A class II

25 molecules, referred to as C2D). Adult male HLA-DR3/DQ2/C2D mice were immunized with 50 µg of FNA or FNA Variant emulsified in Complete Freund's Adjuvant. The immunization was administered intraperitoneally. Two weeks later, the mice received another intraperitoneal immunization of 50 µg FNA or the Variant emulsified in Incomplete Freund's Adjuvant. One week later, the mice were bled via the retro-orbital route, and the serum collected. Serum was assessed for antigen-specific IgG

30 antibodies in a direct ELISA protocol. Briefly, 96 well flat-bottomed EIA plates were coated overnight with 10 µg/ml of denatured FNA. Plates were washed, blocked with 1% Fetal Calf Serum, and serum was titrated out at 1:10 dilutions. The serum was washed out of the wells, and antigen-specific IgG was detected with horse radish peroxidase conjugated anti-mouse IgG. Results are presented as serum dilution versus average optical density (x 1000) in Table 1 and Figure 21.

- 27 -

Table 1

Dilution	FNA	FNA Variant
10	2937.5	88
100	2476	120
1000	1695	103
10000	641.5	80
100000	207	85
1000000	129.5	76
10000000	88.5	85

The results indicated the changes introduced into regions 70-84 and 109-123 significantly
5 reduced the ability of DQ2 transgenic mice to mount a humoral response to the variant and provide a
method for *in vivo* characterization of engineered proteins predicted with the methods of this
invention to show reduced immunogenicity in humans.

- 28 -

SEQUENCE LISTING

<110> Genencor International, Inc.

<120> PROTEINS PRODUCING AN ALTERED IMMUNOGENIC RESPONSE AND
METHODS OF MAKING AND USING THE SAME

<130> 11816-57

<140> CA 2,424,720
<141> 2001-09-23

<150> US 09/768,080
<151> 2001-01-23

<150> US 09/677,822
<151> 2000-10-02

<160> 240

<170> PatentIn Ver. 2.1

<210> 1
<211> 1495
<212> DNA
<213> Bacillus amyloliquefaciens

<220>
<221> mat_peptide
<222> (417)..(1495)

<220>
<221> CDS
<222> (96)..(1244)

<220>
<221> misc_feature
<222> (582)..(584)
<223> The nnn at positions 582 through 584 which in a
preferred embodiment (aat) is to code for
asparagine, but which may also code for proline.

<220>
<221> misc_feature
<222> (585)..(587)
<223> The nnn at positions 585 through 587 which in a
preferred embodiment (cct) is to code for proline,
but which may also code for asparagine.

<220>
<221> misc_feature
<222> (597)..(599)
<223> The nnn at positions 597 to 599 which in a
preferred embodiment (aac) is to code for
asparagine, but which may also code for aspartic acid.

<220>
<221> misc_feature
<222> (678)..(680)
<223> The nnn at positions 678 through 680 which in a
preferred embodiment (gca) is to code for

- 29 -

alanine, but which may also code for serine.

<220>

<221> misc_feature

<222> (681)..(683)

<223> The nnn at positions 681 through 683 which in a preferred embodiment (tca) is to code for serine, but which may also code for alanine.

<220>

<221> misc_feature

<222> (708)..(710)

<223> The nnn at positions 708 through 710 which in a preferred embodiment (gct) is to code for alanine, but which may also code for aspartic acid.

<220>

<221> misc_feature

<222> (711)..(713)

<223> The nnn at positions 711 through 713 which in a preferred embodiment (gac) is to code for aspartic acid, but which may also code for alanine.

<220>

<221> misc_feature

<222> (888)..(890)

<223> The nnn at positions 888 through 890 which in a preferred embodiment (act) is to code for threonine, but which may also code for serine.

<220>

<221> misc_feature

<222> (891)..(893)

<223> The nnn at positions 891 through 893 which in a preferred embodiment (tcc) is to code for serine, but which may also code for threonine.

<220>

<221> misc_feature

<222> (1167)..(1169)

<223> The nnn at positions 1167 through 1169 which in a preferred embodiment (gaa) is to code for glutamic acid, but which may also code for glutamine.

<400> 1

```

ggtctactaa aatattattc catactatac aattaatata cagaataatc tgtctattgg 60
ttattctgca aatgaaaaaa aggagaggat aaaga atg aga ggc aaa aaa gta      113
                               Met Arg Gly Lys Lys Val
                               -105

tgg atc agt ttg ctg ttt gct tta gcg tta atc ttt acg atg gcg ttc      161
Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe
-100                               -95                               -90

ggc agc aca tcc tct gcc cag gcg gca ggg aaa tca aac ggg gaa aag      209
Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys
-85                               -80                               -75                               -70

aaa tat att gtc ggg ttt aaa cag aca atg agc acg atg agc gcc gct      257
Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met Ser Ala Ala

```

-65								-60				-55				
aag Lys	aag Lys	aaa Lys	gat Asp -50	gtc Val	att Ile	tct Ser	gaa Glu	aaa Lys -45	ggc Gly	ggg Gly	aaa Lys	gtg Val	caa Gln -40	aag Lys	caa Gln	305
ttc Phe	aaa Lys	tat Tyr -35	gta Val	gac Asp	gca Ala	gct Ala	tca Ser -30	gct Ala	aca Thr	tta Leu	aac Asn	gaa Glu -25	aaa Lys	gct Ala	gta Val	353
aaa Lys	gaa Glu -20	ttg Leu	aaa Lys	aaa Lys	gac Asp	ccg Pro -15	agc Ser	gtc Val	gct Ala	tac Tyr	gtt Val -10	gaa Glu	gaa Glu	gat Asp	cac His	401
gta Val -5	gca Ala	cat His	gcg Ala	tac Tyr -1	gcg Ala 1	cag Gln	tcc Ser	gtg Val	cct Pro 5	tac Tyr	ggc Gly	gta Val	tca Ser	caa Gln 10	att Ile	449
aaa Lys	gcc Ala	cct Pro	gct Ala 15	ctg Leu	cac His	tct Ser	caa Gln	ggc Gly 20	tac Tyr	act Thr	gga Gly	tca Ser	aat Asn 25	gtt Val	aaa Lys	497
gta Val	gcg Ala	gtt Val 30	atc Ile	gac Asp	agc Ser	ggc Gly	atc Ile 35	gat Asp	tct Ser	tct Ser	cat His	cct Pro 40	gat Asp	tta Leu	aag Lys	545
gta Val	gca Ala 45	ggc Gly	gga Gly	gcc Ala	agc Ser	atg Met 50	gtt Val	cct Pro	tct Ser	gaa Glu	aca Thr 55	nnn Xaa	nnn Xaa	ttc Phe	caa Gln	593
gac Asp 60	nnn Xaa	aac Asn	tct Ser	cac His	gga Gly 65	act Thr	cac His	gtt Val	gcc Ala	ggc Gly 70	aca Thr	gtt Val	gcg Ala	gct Ala	ctt Leu 75	641
aat Asn	aac Asn	tca Ser	atc Ile	ggc Gly 80	gta Val	tta Leu	ggc Gly	gtt Val	gcg Ala	cca Pro	agc Ser	nnn Xaa	nnn Xaa	ctt Leu 90	tac Tyr	689
gct Ala	gta Val	aaa Lys	gtt Val 95	ctc Leu	ggc Gly	nnn Xaa	nnn Xaa	ggc Gly 100	tcc Ser	ggc Gly	caa Gln	tac Tyr	agc Ser 105	tgg Trp	atc Ile	737
att Ile	aac Asn	gga Gly 110	atc Ile	gag Glu	tgg Trp	gcg Ala	atc Ile 115	gca Ala	aac Asn	aat Asn	atg Met	gac Asp 120	gtt Val	att Ile	aac Asn	785
atg Met	agc Ser 125	ctc Leu	ggc Gly	gga Gly	cct Pro	tct Ser 130	ggc Gly	tct Ser	gct Ala	gct Ala	tta Leu 135	aaa Lys	gcg Ala	gca Ala	gtt Val	833
gat Asp 140	aaa Lys	gcc Ala	gtt Val	gca Ala	tcc Ser 145	ggc Gly	gtc Val	gta Val	gtc Val	gtt Val 150	gcg Ala	gca Ala	gcc Ala	ggc Gly 155	aac Asn	881
gaa Glu	ggc Gly	nnn Xaa	nnn Xaa	ggc Gly 160	agc Ser	tca Ser	agc Ser	aca Thr	gtg Val 165	ggc Gly	tac Tyr	cct Pro	ggc Gly 170	aaa Lys	tac Tyr	929
cct Pro	tct Ser	gtc Val	att Ile 175	gca Ala	gta Val	ggc Gly	gct Ala	gtt Val 180	gac Asp	agc Ser	agc Ser	aac Asn	caa Gln 185	aga Arg	gca Ala	977

- 31 -

```

tct ttc tca agc gta gga cct gag ctt gat gtc atg gca cct ggc gta 1025
Ser Phe Ser Ser Val Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val
      190                      195                      200

tct atc caa agc acg ctt cct gga aac aaa tac ggg gcg tac aac ggt 1073
Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly
      205                      210                      215

acg tca atg gca tct ccg cac gtt gcc gga gcg gct gct ttg att ctt 1121
Thr Ser Met Ala Ser Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu
220                      225                      230                      235

tct aag cac ccg aac tgg aca aac act caa gtc cgc agc agt tta nnn 1169
Ser Lys His Pro Asn Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Xaa
      240                      245                      250

aac acc act aca aaa ctt ggt gat tct ttc tac tat gga aaa ggg ctg 1217
Asn Thr Thr Thr Lys Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu
      255                      260                      265

atc aac gta cag gcg gca gct cag taa aacataaaaa accggccttg 1264
Ile Asn Val Gln Ala Ala Ala Gln
      270                      275

gccccgccgg tttttttatt tttcttctc cgcattgttca atccgctcca taatcgacgg 1324

atggctccct ctgaaaattt taacgagaaa cggcggggtg acccggtcca gtcccgtaac 1384

ggccaagtcc tgaaacgtct caatcgccgc ttcccgggtt ccggtcagct caatgccgta 1444

acggtcggcg gcgttttctt gataccggga gacggcattc gtaatcggat c 1495

```

```

<210> 2
<211> 382
<212> PRT
<213> Bacillus amyloliquefaciens

```

```

<220>
<221> VARIANT
<222> (163)...(163)
<223> Xaa = Asn or Pro

```

```

<220>
<221> VARIANT
<222> (164)...(164)
<223> Xaa = Pro or Asn

```

```

<220>
<221> VARIANT
<222> (167)...(167)
<223> Xaa = Asn or Asp

```

```

<220>
<221> VARIANT
<222> (195)...(195)
<223> Xaa = Ala or Ser

```

```

<220>
<221> VARIANT

```

- 32 -

<222> (196)...(196)
 <223> Xaa = Ser or Ala

<220>
 <221> VARIANT
 <222> (205)...(205)
 <223> Xaa = Ala or Asp

<220>
 <221> VARIANT
 <222> (206)...(206)
 <223> Xaa = Asp or Ala

<220>
 <221> VARIANT
 <222> (265)...(265)
 <223> Xaa = Thr or Ser

<220>
 <221> VARIANT
 <222> (266)...(266)
 <223> Xaa = Ser or Thr

<220>
 <221> VARIANT
 <222> (358)...(358)
 <223> Xaa = Gln or Glu

<400> 2

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

- 33 -

```

Val Ala Ala Ala Gly Asn Glu Gly Xaa Xaa Gly Ser Ser Ser Thr Val
      260      265      270
Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp
      275      280      285
Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu Asp
      290      295      300
Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys
305      310      315      320
Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val Ala Gly
      325      330      335
Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr Gln
      340      345      350
Val Arg Ser Ser Leu Xaa Asn Thr Thr Thr Lys Leu Gly Asp Ser Phe
      355      360      365
Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
      370      375      380

```

```

<210> 3
<211> 275
<212> PRT
<213> Bacillus amyloliquefaciens

```

```

<400> 3
Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
  1      5      10      15

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

Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
      35      40      45

Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
      50      55      60

Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
      65      70      75      80

Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
      85      90      95

Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
      100      105      110

Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
      115      120      125

Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
      130      135      140

Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
      145      150      155      160

Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
      165      170      175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
      180      185      190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr

```

- 34 -

195	200	205
Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser		
210	215	220
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn		
225	230	235
Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys		
	245	250
Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala		
	260	265
		270
Ala Ala Gln		
275		

<210> 4

<211> 275

<212> PRT

<213> Bacillus subtilis

<400> 4

Ala Gln Ser Val Pro Tyr Gly Ile Ser Gln Ile Lys Ala Pro Ala Leu		
1	5	10
		15
His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp		
	20	25
		30
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala		
	35	40
		45
Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His		
	50	55
		60
Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly		
65	70	75
		80
Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu		
	85	90
		95
Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu		
	100	105
		110
Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly		
	115	120
		125
Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser		
	130	135
		140
Ser Gly Ile Val Val Ala Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly		
145	150	155
		160
Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala		
	165	170
		175
Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala		
	180	185
		190
Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr		

- 35 -

195	200	205
Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr		
210	215	220
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr		
225	230	235
Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr		
	245	250
		255
Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala		
	260	265
		270
Ala Ala Gln		
275		

<210> 5

<211> 274

<212> PRT

<213> Bacillus licheniformis

<400> 5

Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
1 5 10 15
Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp
20 25 30
Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala
35 40 45
Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly
50 55 60
Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val
65 70 75 80
Leu Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn
85 90 95
Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp
100 105 110
Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala
115 120 125
Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg
130 135 140
Gly Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Asn Ser Gly Ser
145 150 155 160
Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val
165 170 175
Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly
180 185 190
Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr

- 36 -

195	200	205
Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro		
210	215	220
His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu		
225	230	235
Ser Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu		
	245	250
Gly Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala		
	260	265
		270

Ala Gln

<210> 6
 <211> 269
 <212> PRT
 <213> Bacillus lentus

<400> 6
Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15
His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30
Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45
Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60
His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80
Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95
Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110
Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125
Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140
Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145 150 155 160
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175
Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190
Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr

- 37 -

195		200		205
Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala				
210		215		220
Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile				
225		230		235
				240
Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu				
	245		250	255
Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg				
	260		265	

<210> 7
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 7
Ile Lys Asp Phe His Val Tyr Phe Arg Glu Ser Arg Asp Ala Gly
1 5 10 15

<210> 8
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 8
Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly Val Leu Val
1 5 10 15

<210> 9
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 9
Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala
1 5 10 15

<210> 10
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 38 -

<400> 10

Val	Pro	Trp	Gly	Ile	Ser	Arg	Val	Gln	Ala	Pro	Ala	Ala	His	Asn
1				5				10						15

<210> 11

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 11

Gly	Ile	Ser	Arg	Val	Gln	Ala	Pro	Ala	Ala	His	Asn	Arg	Gly	Leu
1				5				10						15

<210> 12

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 12

Arg	Val	Gln	Ala	Pro	Ala	Ala	His	Asn	Arg	Gly	Leu	Thr	Gly	Ser
1				5				10						15

<210> 13

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 13

Ala	Pro	Ala	Ala	His	Asn	Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys
1				5				10						15

<210> 14

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 14

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

<210> 15

<211> 15

<212> PRT

- 39 -

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 15

Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp	Thr
1				5				10						15

<210> 16

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 16

Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp	Thr	Gly	Ile	Ser
1				5					10					15

<210> 17

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 17

Gly	Val	Lys	Val	Ala	Val	Leu	Asp	Thr	Gly	Ile	Ser	Thr	His	Pro
1				5					10					15

<210> 18

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 18

Val	Ala	Val	Leu	Asp	Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn
1				5					10					15

<210> 19

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 19

Leu	Asp	Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly
1				5					10					15

- 40 -

<210> 20
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 20
 Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 1 5 10 15

<210> 21
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 21
 Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser Phe Val Pro
 1 5 10 15

<210> 22
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 22
 Asp Leu Asn Ile Arg Gly Gly Ala Ser Phe Val Pro Gly Glu Pro
 1 5 10 15

<210> 23
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 23
 Ile Arg Gly Gly Ala Ser Phe Val Pro Gly Glu Pro Ser Thr Gln
 1 5 10 15

<210> 24
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 41 -

<400> 24

Gly	Ala	Ser	Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn
1				5				10						15

<210> 25

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 25

Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly
1				5				10						15

<210> 26

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 26

Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr	His	Val
1				5				10						15

<210> 27

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 27

Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr	His	Val	Ala	Gly	Thr
1				5				10						15

<210> 28

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 28

Asp	Gly	Asn	Gly	His	Gly	Thr	His	Val	Ala	Gly	Thr	Ile	Ala	Ala
1				5				10						15

<210> 29

<211> 15

<212> PRT

<213> Artificial Sequence

- 42 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 29

Gly	His	Gly	Thr	His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn
1				5					10					15

<210> 30

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 30

Thr	His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly
1				5					10					15

<210> 31

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 31

Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu	Gly
1				5					10					15

<210> 32

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 32

Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu	Gly	Val	Ala	Pro
1				5					10					15

<210> 33

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 33

Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Glu
1				5					10					15

- 43 -

<210> 34
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 34
 Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala
 1 5 10 15

<210> 35
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 35
 Val Leu Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val
 1 5 10 15

<210> 36
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 36
 Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 1 5 10 15

<210> 37
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 37
 Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala Ser Gly Ser
 1 5 10 15

<210> 38
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 38

- 44 -

Leu Tyr Ala Val Lys Val Leu Gly Ala Ser Gly Ser Gly Ser Val
 1 5 10 15

<210> 39
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 39
 Val Lys Val Leu Gly Ala Ser Gly Ser Gly Ser Val Ser Ser Ile
 1 5 10 15

<210> 40
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 40
 Leu Gly Ala Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly
 1 5 10 15

<210> 41
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 41
 Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp
 1 5 10 15

<210> 42
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 42
 Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala Gly Asn
 1 5 10 15

<210> 43
 <211> 15
 <212> PRT
 <213> Artificial Sequence

- 45 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 43

Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala	Gly	Asn	Asn	Gly	Met
1				5					10					15

<210> 44

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 44

Ala	Gln	Gly	Leu	Glu	Trp	Ala	Gly	Asn	Asn	Gly	Met	His	Val	Ala
1				5					10					15

<210> 45

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 45

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

<210> 46

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 46

Ala	Gly	Asn	Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser
1				5					10					15

<210> 47

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 47

Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser	Pro	Ser	Pro
1				5					10					15

<210> 48

- 46 -

<211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 48
 His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser Pro Ser Ala Thr
 1 5 10 15

<210> 49
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 49
 Asn Leu Ser Leu Gly Ser Pro Ser Pro Ser Ala Thr Leu Glu Gln
 1 5 10 15

<210> 50
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 50
 Leu Gly Ser Pro Ser Pro Ser Ala Thr Leu Glu Gln Ala Val Asn
 1 5 10 15

<210> 51
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 51
 Pro Ser Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr
 1 5 10 15

<210> 52
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 52
 Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly

- 47 -

1	5	10	15
---	---	----	----

<210> 53
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 53
 Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly Val Leu Val
 1 5 10 15

<210> 54
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 54
 Ala Val Asn Ser Ala Thr Ser Arg Gly Val Leu Val Val Ala Ala
 1 5 10 15

<210> 55
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 55
 Ser Ala Thr Ser Arg Gly Val Leu Val Val Ala Ala Ser Gly Asn
 1 5 10 15

<210> 56
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 56
 Ser Arg Gly Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala
 1 5 10 15

<210> 57
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

- 48 -

<223> Description of Artificial Sequence: Synthetic

<400> 57

Val	Leu	Val	Val	Ala	Ala	Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile
1				5					10					15

<210> 58

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 58

Val	Ala	Ala	Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile	Ser	Tyr	Pro
1				5				10						15

<210> 59

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 59

Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr
1				5				10						15

<210> 60

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 60

Ser	Gly	Ala	Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala
1				5				10						15

<210> 61

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 61

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5				10						15

<210> 62

<211> 15

- 49 -

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 62

Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr
1				5					10					15

<210> 63

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 63

Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr	Asp	Gln	Asn
1				5					10					15

<210> 64

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 64

Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr	Asp	Gln	Asn	Asn	Asn	Arg
1				5					10					15

<210> 65

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 65

Met	Ala	Val	Gly	Ala	Thr	Asp	Gln	Asn	Asn	Asn	Arg	Ala	Ser	Phe
1				5					10					15

<210> 66

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 66

Gly	Ala	Thr	Asp	Gln	Asn	Asn	Asn	Arg	Ala	Ser	Phe	Ser	Gln	Tyr
1				5					10					15

- 50 -

<210> 67
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 67
 Asp Gln Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly
 1 5 10 15

<210> 68
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 68
 Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 1 5 10 15

<210> 69
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 69
 Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile Val Ala Pro
 1 5 10 15

<210> 70
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 70
 Ser Gln Tyr Gly Ala Gly Leu Asp Ile Val Ala Pro Gly Val Asn
 1 5 10 15

<210> 71
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 51 -

<400> 71

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

<210> 72

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 72

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

<210> 73

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 73

Val	Ala	Pro	Gly	Val	Asn	Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr
1				5				10						15

<210> 74

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 74

Gly	Val	Asn	Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr	Tyr	Ala	Ser
1				5					10					15

<210> 75

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 75

Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr	Tyr	Ala	Ser	Leu	Asn	Gly
1				5					10					15

<210> 76

<211> 15

<212> PRT

- 52 -

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 76

Thr	Tyr	Pro	Gly	Ser	Thr	Tyr	Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met
1				5					10					15

<210> 77

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 77

Gly	Ser	Thr	Tyr	Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro
1				5					10					15

<210> 78

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 78

Tyr	Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro	His	Val	Ala
1				5					10					15

<210> 79

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 79

Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro	His	Val	Ala	Gly	Ala	Ala
1				5					10					15

<210> 80

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 80

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

- 53 -

<210> 81
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 81
Ala Thr Pro His Val Ala Gly Ala Ala Ala Leu Val Lys Gln Lys
1 5 10 15

<210> 82
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 82
Gly Val Ala Gly Ala Ala Ala Leu Val Lys Gln Lys Asn Pro Ser
1 5 10 15

<210> 83
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 83
Gly Ala Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn
1 5 10 15

<210> 84
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 84
Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
1 5 10 15

<210> 85
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

- 54 -

<400> 85

Lys	Gln	Lys	Asn	Pro	Ser	Trp	Ser	Val	Asn	Gln	Ile	Arg	Asn	His
1				5					10					15

<210> 86

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 86

Asn	Pro	Ser	Trp	Ser	Asn	Val	Gln	Ile	Arg	Asn	His	Leu	Lys	Asn
1				5					10					15

<210> 87

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 87

Trp	Ser	Asn	Val	Gln	Ile	Arg	Asn	His	Leu	Lys	Asn	Thr	Ala	Thr
1				5					10					15

<210> 88

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 88

Val	Gln	Ile	Arg	Asn	His	Leu	Lys	Asn	Thr	Ala	Thr	Ser	Leu	Gly
1				5					10					15

<210> 89

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 89

Arg	Asn	His	Leu	Lys	Asn	Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn
1				5					10					15

<210> 90

<211> 15

<212> PRT

<213> Artificial Sequence

- 55 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 90

Leu	Lys	Asn	Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn	Leu	Tyr	Gly
1				5				10						15

<210> 91

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 91

Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn	Leu	Tyr	Gly	Ser	Gly	Leu
1				5				10						15

<210> 92

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 92

Ser	Leu	Gly	Ser	Thr	Asn	Leu	Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala
1				5				10						15

<210> 93

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 93

Ser	Thr	Asn	Leu	Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala	Glu	Ala	Ala
1				5				10						15

<210> 94

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 94

Asn	Leu	Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala	Glu	Ala	Ala	Thr	Arg
1				5				10						15

- 56 -

<210> 95
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 95
 Asp Ala Glu Leu His Ile Phe Arg Val Phe Thr Asn Asn Gln Val
 1 5 10 15

<210> 96
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 96
 Pro Leu Arg Arg Ala Ser Leu Ser Leu Gly Ser Gly Phe Trp His
 1 5 10 15

<210> 97
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 97
 Arg Ala Ser Leu Ser Leu Gly Ser Gly Phe Trp His Ala Thr Gly
 1 5 10 15

<210> 98
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 98
 Leu Ser Leu Gly Ser Gly Phe Trp His Ala Thr Gly Arg His Ser
 1 5 10 15

<210> 99
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 99

- 57 -

Gly	Ser	Gly	Phe	Trp	His	Ala	Thr	Gly	Arg	His	Ser	Ser	Arg	Arg
1				5					10					15

<210> 100
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

Phe	Trp	His	Ala	Thr	Gly	Arg	His	Ser	Ser	Arg	Arg	Leu	Leu	Arg
1				5					10					15

<210> 101
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

Ala	Thr	Gly	Arg	His	Ser	Ser	Arg	Arg	Leu	Leu	Arg	Ala	Ile	Pro
1				5					10					15

<210> 102
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

Arg	His	Ser	Ser	Arg	Arg	Leu	Leu	Arg	Ala	Ile	Pro	Arg	Gln	Val
1				5					10					15

<210> 103
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

Ser	Arg	Arg	Leu	Leu	Arg	Ala	Ile	Pro	Arg	Gln	Val	Ala	Gln	Thr
1				5					10					15

<210> 104
 <211> 15
 <212> PRT
 <213> Artificial Sequence

- 58 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 104

Leu	Leu	Arg	Ala	Ile	Pro	Arg	Gln	Val	Ala	Gln	Thr	Leu	Gln	Ala
1				5					10					15

<210> 105

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 105

Ala	Ile	Pro	Arg	Gln	Val	Ala	Gln	Thr	Leu	Gln	Ala	Asp	Val	Leu
1				5					10					15

<210> 106

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 106

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

<210> 107

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 107

Ala	Gln	Thr	Leu	Gln	Ala	Asp	Val	Leu	Trp	Gln	Met	Gly	Tyr	Thr
1				5					10					15

<210> 108

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 108

Leu	Gln	Ala	Asp	Val	Leu	Trp	Gln	Met	Gly	Tyr	Thr	Gly	Ala	Asn
1				5					10					15

<210> 109

- 59 -

<211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 109

Asp	Val	Leu	Trp	Gln	Met	Gly	Tyr	Thr	Gly	Ala	Asn	Val	Arg	Val
1				5					10					15

<210> 110

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 110

Trp	Gln	Met	Gly	Tyr	Thr	Gly	Ala	Asn	Val	Arg	Val	Ala	Val	Phe
1				5					10					15

<210> 111

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 111

Gly	Tyr	Thr	Gly	Ala	Asn	Val	Arg	Val	Ala	Val	Phe	Asp	Thr	Gly
1				5					10					15

<210> 112

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 112

Gly	Ala	Asn	Val	Arg	Val	Ala	Val	Phe	Asp	Thr	Gly	Leu	Ser	Glu
1				5					10					15

<210> 113

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 113

Val	Arg	Val	Ala	Val	Phe	Asp	Thr	Gly	Leu	Ser	Glu	Lys	His	Pro
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

- 60 -

1	5	10	15
---	---	----	----

<210> 114
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 114
 Ala Val Phe Asp Thr Gly Leu Ser Glu Lys His Pro His Phe Lys
 1 5 10 15

<210> 115
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 115
 Asp Thr Gly Leu Ser Glu Lys His Pro His Phe Lys Asn Val Lys
 1 5 10 15

<210> 116
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 116
 Leu Ser Glu Lys His Pro His Phe Lys Asn Val Lys Glu Arg Thr
 1 5 10 15

<210> 117
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 117
 Lys His Pro His Phe Lys Asn Val Lys Glu Arg Thr Asn Trp Thr
 1 5 10 15

<210> 118
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

- 61 -

<223> Description of Artificial Sequence: Synthetic

<400> 118

His	Phe	Lys	Asn	Val	Lys	Glu	Arg	Thr	Asn	Trp	Thr	Asn	Glu	Arg
1				5				10					15	

<210> 119

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 119

Asn	Val	Lys	Glu	Arg	Thr	Asn	Trp	Thr	Asn	Glu	Arg	Thr	Leu	Asp
1				5				10					15	

<210> 120

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 120

Glu	Arg	Thr	Asn	Trp	Thr	Asn	Glu	Arg	Thr	Leu	Asp	Asp	Gly	Leu
1				5				10					15	

<210> 121

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 121

Asn	Trp	Thr	Asn	Glu	Arg	Thr	Leu	Asp	Asp	Gly	Leu	Gly	His	Gly
1				5				10					15	

<210> 122

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 122

Asn	Glu	Arg	Thr	Leu	Asp	Asp	Gly	Leu	Gly	His	Gly	Thr	Phe	Val
1				5				10					15	

<210> 123

<211> 15

- 62 -

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 123

Thr	Leu	Asp	Asp	Gly	Leu	Gly	His	Gly	Thr	Phe	Val	Ala	Gly	Val
1				5				10					15	

<210> 124

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 124

Asp	Gly	Leu	Gly	His	Gly	Thr	Phe	Val	Ala	Gly	Val	Ile	Ala	Ser
1				5				10					15	

<210> 125

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 125

Gly	His	Gly	Thr	Phe	Val	Ala	Gly	Val	Ile	Ala	Ser	Met	Arg	Glu
1				5				10					15	

<210> 126

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 126

Thr	Phe	Val	Ala	Gly	Val	Ile	Ala	Ser	Met	Arg	Glu	Cys	Gln	Gly
1				5				10					15	

<210> 127

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 127

Ala	Gly	Val	Ile	Ala	Ser	Met	Arg	Glu	Cys	Gln	Gly	Phe	Ala	Pro
1				5				10					15	

- 63 -

<210> 128
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 128
 Ile Ala Ser Met Arg Glu Cys Gln Gly Phe Ala Pro Asp Ala Glu
 1 5 10 15

<210> 129
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 129
 Met Arg Glu Cys Gln Gly Phe Ala Pro Asp Ala Glu Leu His Ile
 1 5 10 15

<210> 130
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 130
 Cys Gln Gly Phe Ala Pro Asp Ala Glu Leu His Ile Phe Arg Val
 1 5 10 15

<210> 131
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 131
 Phe Ala Pro Asp Ala Glu Leu His Ile Phe Arg Val Phe Thr Asn
 1 5 10 15

<210> 132
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 64 -

<400> 132

Asp	Ala	Glu	Leu	His	Ile	Phe	Arg	Val	Phe	Thr	Asn	Asn	Gln	Val
1				5					10					15

<210> 133

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 133

Leu	His	Ile	Phe	Arg	Val	Phe	Thr	Asn	Asn	Gln	Val	Ser	Tyr	Thr
1				5				10						15

<210> 134

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 134

Phe	Arg	Val	Phe	Thr	Asn	Asn	Gln	Val	Ser	Tyr	Thr	Ser	Trp	Phe
1				5					10					15

<210> 135

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 135

Phe	Thr	Asn	Asn	Gln	Val	Ser	Tyr	Thr	Ser	Trp	Phe	Leu	Asp	Ala
1				5					10					15

<210> 136

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 136

Asn	Gln	Val	Ser	Tyr	Thr	Ser	Trp	Phe	Leu	Asp	Ala	Phe	Asn	Tyr
1				5					10					15

<210> 137

<211> 15

<212> PRT

- 65 -

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 137

Ser	Tyr	Thr	Ser	Trp	Phe	Leu	Asp	Ala	Phe	Asn	Tyr	Ala	Ile	Leu
1				5					10					15

<210> 138

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 138

Ser	Trp	Phe	Leu	Asp	Ala	Phe	Asn	Tyr	Ala	Ile	Leu	Lys	Lys	Ile
1				5					10					15

<210> 139

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 139

Leu	Asp	Ala	Phe	Asn	Tyr	Ala	Ile	Leu	Lys	Lys	Ile	Asp	Val	Leu
1				5					10					15

<210> 140

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 140

Phe	Asn	Tyr	Ala	Ile	Leu	Lys	Lys	Ile	Asp	Val	Leu	Asn	Leu	Ser
1				5					10					15

<210> 141

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 141

Ala	Ile	Leu	Lys	Lys	Ile	Asp	Val	Leu	Asn	Leu	Ser	Ile	Gly	Gly
1				5					10					15

- 66 -

<210> 142
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 142
Lys Lys Ile Asp Val Leu Asn Leu Ser Ile Gly Gly Pro Asp Phe
1 5 10 15

<210> 143
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 143
Asp Val Leu Asn Leu Ser Ile Gly Gly Pro Asp Phe Met Asp His
1 5 10 15

<210> 144
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 144
Asn Leu Ser Ile Gly Gly Pro Asp Phe Met Asp His Pro Phe Val
1 5 10 15

<210> 145
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 145
Ile Gly Gly Pro Asp Phe Met Asp His Pro Phe Val Asp Lys Val
1 5 10 15

<210> 146
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

- 67 -

<400> 146

Pro	Asp	Phe	Met	Asp	His	Pro	Phe	Val	Asp	Lys	Val	Trp	Glu	Leu
1				5					10					15

<210> 147

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 147

Met	Asp	His	Pro	Phe	Val	Asp	Lys	Val	Trp	Glu	Leu	Thr	Ala	Asn
1				5					10					15

<210> 148

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 148

Pro	Phe	Val	Asp	Lys	Val	Trp	Glu	Leu	Thr	Ala	Asn	Asn	Val	Ile
1				5					10					15

<210> 149

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 149

Asp	Lys	Val	Trp	Glu	Leu	Thr	Ala	Asn	Asn	Val	Ile	Met	Val	Ser
1				5					10					15

<210> 150

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 150

Trp	Glu	Leu	Thr	Ala	Asn	Asn	Val	Ile	Met	Val	Ser	Ala	Ile	Gly
1				5					10					15

<210> 151

<211> 15

<212> PRT

<213> Artificial Sequence

- 68 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 151

Thr	Ala	Asn	Asn	Val	Ile	Met	Val	Ser	Ala	Ile	Gly	Asn	Asp	Gly
1				5					10					15

<210> 152

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 152

Asn	Val	Ile	Met	Val	Ser	Ala	Ile	Gly	Asn	Asp	Gly	Pro	Leu	Tyr
1				5					10					15

<210> 153

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 153

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

<210> 154

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 154

Ala	Ile	Gly	Asn	Asp	Gly	Pro	Leu	Tyr	Gly	Thr	Leu	Asn	Asn	Pro
1				5					10					15

<210> 155

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 155

Asn	Asp	Gly	Pro	Leu	Tyr	Gly	Thr	Leu	Asn	Asn	Pro	Ala	Asp	Gln
1				5					10					15

- 69 -

<210> 156
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 156
 Pro Leu Tyr Gly Thr Leu Asn Asn Pro Ala Asp Gln Met Asp Val
 1 5 10 15

<210> 157
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 157
 Gly Thr Leu Asn Asn Pro Ala Asp Gln Met Asp Val Ile Gly Val
 1 5 10 15

<210> 158
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 158
 Asn Asn Pro Ala Asp Gln Met Asp Val Ile Gly Val Gly Gly Ile
 1 5 10 15

<210> 159
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 159
 Ala Asp Gln Met Asp Val Ile Gly Val Gly Gly Ile Asp Phe Glu
 1 5 10 15

<210> 160
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 160

- 70 -

Met	Asp	Val	Ile	Gly	Val	Gly	Gly	Ile	Asp	Phe	Glu	Asp	Asn	Ile
1				5					10					15

<210> 161

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 161

Ile	Gly	Val	Gly	Gly	Ile	Asp	Phe	Glu	Asp	Asn	Ile	Ala	Arg	Phe
1				5					10					15

<210> 162

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 162

Gly	Gly	Ile	Asp	Phe	Glu	Asp	Asn	Ile	Ala	Arg	Phe	Ser	Ser	Arg
1				5					10					15

<210> 163

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 163

Asp	Phe	Glu	Asp	Asn	Ile	Ala	Arg	Phe	Ser	Ser	Arg	Gly	Met	Thr
1				5					10					15

<210> 164

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 164

Asp	Asn	Ile	Ala	Arg	Phe	Ser	Ser	Arg	Gly	Met	Thr	Thr	Trp	Glu
1				5					10					15

<210> 165

<211> 15

<212> PRT

<213> Artificial Sequence

- 71 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 165

Ala	Arg	Phe	Ser	Ser	Arg	Gly	Met	Thr	Thr	Trp	Glu	Leu	Pro	Gly
1				5					10					15

<210> 166

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 166

Ser	Ser	Arg	Gly	Met	Thr	Thr	Trp	Glu	Leu	Pro	Gly	Gly	Tyr	Gly
1				5					10					15

<210> 167

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 167

Gly	Met	Thr	Thr	Trp	Glu	Leu	Pro	Gly	Gly	Tyr	Gly	Arg	Met	Lys
1				5					10					15

<210> 168

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 168

Thr	Trp	Glu	Leu	Pro	Gly	Gly	Tyr	Gly	Arg	Met	Lys	Pro	Asp	Ile
1				5					10					15

<210> 169

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 169

Leu	Pro	Gly	Gly	Tyr	Gly	Arg	Met	Lys	Pro	Asp	Ile	Val	Thr	Tyr
1				5					10					15

<210> 170

- 72 -

<211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 170
 Gly Tyr Gly Arg Met Lys Pro Asp Ile Val Thr Tyr Gly Ala Gly
 1 5 10 15

<210> 171
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 171
 Arg Met Lys Pro Asp Ile Val Thr Tyr Gly Ala Gly Val Arg Gly
 1 5 10 15

<210> 172
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 172
 Pro Asp Ile Val Thr Tyr Gly Ala Gly Val Arg Gly Ser Gly Val
 1 5 10 15

<210> 173
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 173
 Val Thr Tyr Gly Ala Gly Val Arg Gly Ser Gly Val Lys Gly Gly
 1 5 10 15

<210> 174
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 174
 Gly Ala Gly Val Arg Gly Ser Gly Val Lys Gly Gly Cys Arg Ala

- 73 -

1	5	10	15
---	---	----	----

<210> 175
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 175
 Val Arg Gly Ser Gly Val Lys Gly Gly Cys Arg Ala Leu Ser Gly
 1 5 10 15

<210> 176
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 176
 Ser Gly Val Lys Gly Gly Cys Arg Ala Leu Ser Gly Thr Ser Val
 1 5 10 15

<210> 177
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 177
 Lys Gly Gly Cys Arg Ala Leu Ser Gly Thr Ser Val Ala Ser Pro
 1 5 10 15

<210> 178
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 178
 Cys Arg Ala Leu Ser Gly Thr Ser Val Ala Ser Pro Val Val Ala
 1 5 10 15

<210> 179
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

- 74 -

<223> Description of Artificial Sequence: Synthetic

<400> 179

Leu	Ser	Gly	Thr	Ser	Val	Ala	Ser	Pro	Val	Val	Ala	Gly	Ala	Val
1				5					10					15

<210> 180

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 180

Thr	Ser	Val	Ala	Ser	Pro	Val	Val	Ala	Gly	Ala	Val	Thr	Leu	Leu
1				5					10					15

<210> 181

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 181

Ala	Ser	Pro	Val	Val	Ala	Gly	Ala	Val	Thr	Leu	Leu	Val	Ser	Thr
1				5					10					15

<210> 182

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 182

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

<210> 183

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 183

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

<210> 184

<211> 15

- 75 -

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 184

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

<210> 185

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 185

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

<210> 186

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 186

Val	Gln	Lys	Arg	Glu	Leu	Val	Asn	Pro	Ala	Ser	Met	Lys	Gln	Ala
1				5					10					15

<210> 187

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 187

Arg	Glu	Leu	Val	Asn	Pro	Ala	Ser	Met	Lys	Gln	Ala	Leu	Ile	Ala
1				5					10					15

<210> 188

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 188

Val	Asn	Pro	Ala	Ser	Met	Lys	Gln	Ala	Leu	Ile	Ala	Ser	Ala	Arg
1				5					10					15

- 76 -

<210> 189
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 189
 Ala Ser Met Lys Gln Ala Leu Ile Ala Ser Ala Arg Arg Leu Pro
 1 5 10 15

<210> 190
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 190
 Lys Gln Ala Leu Ile Ala Ser Ala Arg Arg Leu Pro Gly Val Asn
 1 5 10 15

<210> 191
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 191
 Leu Ile Ala Ser Ala Arg Arg Leu Pro Gly Val Asn Met Phe Glu
 1 5 10 15

<210> 192
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 192
 Ser Ala Arg Arg Leu Pro Gly Val Asn Met Phe Glu Gln Gly His
 1 5 10 15

<210> 193
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 77 -

<400> 193

Arg	Leu	Pro	Gly	Val	Asn	Met	Phe	Glu	Gln	Gly	His	Gly	Lys	Leu
1				5					10					15

<210> 194

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 194

Gly	Val	Asn	Met	Phe	Glu	Gln	Gly	His	Gly	Lys	Leu	Asp	Leu	Leu
1				5					10					15

<210> 195

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 195

Met	Phe	Glu	Gln	Gly	His	Gly	Lys	Leu	Asp	Leu	Leu	Arg	Ala	Tyr
1				5					10					15

<210> 196

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 196

Gln	Gly	His	Gly	Lys	Leu	Asp	Leu	Leu	Arg	Ala	Tyr	Gln	Ile	Leu
1				5					10					15

<210> 197

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 197

Gly	Lys	Leu	Asp	Leu	Leu	Arg	Ala	Tyr	Gln	Ile	Leu	Asn	Ser	Tyr
1				5					10					15

<210> 198

<211> 15

<212> PRT

- 78 -

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 198

Asp	Leu	Leu	Arg	Ala	Tyr	Gln	Ile	Leu	Asn	Ser	Tyr	Lys	Pro	Gln
1				5					10					15

<210> 199

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 199

Arg	Ala	Tyr	Gln	Ile	Leu	Asn	Ser	Tyr	Lys	Pro	Gln	Ala	Ser	Leu
1				5					10					15

<210> 200

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 200

Gln	Ile	Leu	Asn	Ser	Tyr	Lys	Pro	Gln	Ala	Ser	Leu	Ser	Pro	Ser
1				5					10					15

<210> 201

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 201

Asn	Ser	Tyr	Lys	Pro	Gln	Ala	Ser	Leu	Ser	Pro	Ser	Tyr	Ile	Asp
1				5					10					15

<210> 202

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 202

Lys	Pro	Gln	Ala	Ser	Leu	Ser	Pro	Ser	Tyr	Ile	Asp	Leu	Thr	Glu
1				5					10					15

- 79 -

<210> 203
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 203
 Ala Ser Leu Ser Pro Ser Tyr Ile Asp Leu Thr Glu Cys Pro Tyr
 1 5 10 15

<210> 204
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 204
 Ser Pro Ser Tyr Ile Asp Leu Thr Glu Cys Pro Tyr Met Trp Pro
 1 5 10 15

<210> 205
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 205
 Tyr Ile Asp Leu Thr Glu Cys Pro Tyr Met Trp Pro Tyr Cys Ser
 1 5 10 15

<210> 206
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

<400> 206
 Leu Thr Glu Cys Pro Tyr Met Trp Pro Tyr Cys Ser Gln Pro Ile
 1 5 10 15

<210> 207
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

- 80 -

<400> 207

Cys	Pro	Tyr	Met	Trp	Pro	Tyr	Cys	Ser	Gln	Pro	Ile	Tyr	Tyr	Gly
1				5					10					15

<210> 208

<211> 1052

<212> PRT

<213> Homo sapiens

<400> 208

Met	Lys	Leu	Val	Asn	Ile	Trp	Leu	Leu	Leu	Leu	Val	Val	Leu	Leu	Cys
1				5					10					15	

Gly	Lys	Lys	His	Leu	Gly	Asp	Arg	Leu	Glu	Lys	Lys	Ser	Phe	Glu	Lys
			20					25					30		

Ala	Pro	Cys	Pro	Gly	Cys	Ser	His	Leu	Thr	Leu	Lys	Val	Glu	Phe	Ser
		35					40					45			

Ser	Thr	Val	Val	Glu	Tyr	Glu	Tyr	Ile	Val	Ala	Phe	Asn	Gly	Tyr	Phe
	50					55					60				

Thr	Ala	Lys	Ala	Arg	Asn	Ser	Phe	Ile	Ser	Ser	Ala	Leu	Lys	Ser	Ser
65					70					75					80

Glu	Val	Asp	Asn	Trp	Arg	Ile	Ile	Pro	Arg	Asn	Asn	Pro	Ser	Ser	Asp
			85						90					95	

Tyr	Pro	Ser	Asp	Phe	Glu	Val	Ile	Gln	Ile	Lys	Glu	Lys	Gln	Lys	Ala
			100					105					110		

Gly	Leu	Leu	Thr	Leu	Glu	Asp	His	Pro	Asn	Ile	Lys	Arg	Val	Thr	Pro
	115						120					125			

Gln	Arg	Lys	Val	Phe	Arg	Ser	Leu	Lys	Tyr	Ala	Glu	Ser	Asp	Pro	Thr
130						135					140				

Val	Pro	Cys	Asn	Glu	Thr	Arg	Trp	Ser	Gln	Lys	Trp	Gln	Ser	Ser	Arg
145					150					155					160

Pro	Leu	Arg	Arg	Ala	Ser	Leu	Ser	Leu	Gly	Ser	Gly	Phe	Trp	His	Ala
				165					170					175	

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

Val	Ala	Gln	Thr	Leu	Gln	Ala	Asp	Val	Leu	Trp	Gln	Met	Gly	Tyr	Thr
		195					200					205			

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

His	Pro	His	Phe	Lys	Asn	Val	Lys	Glu	Arg	Thr	Asn	Trp	Thr	Asn	Glu
225					230					235					240

Arg	Thr	Leu	Asp	Asp	Gly	Leu	Gly	His	Gly	Thr	Phe	Val	Ala	Gly	Val
				245					250					255	

Ile	Ala	Ser	Met	Arg	Glu	Cys	Gln	Gly	Phe	Ala	Pro	Asp	Ala	Glu	Leu
			260					265					270		

- 81 -

His	Ile	Phe	Arg	Val	Phe	Thr	Asn	Asn	Gln	Val	Ser	Tyr	Thr	Ser	Trp
		275					280					285			
Phe	Leu	Asp	Ala	Phe	Asn	Tyr	Ala	Ile	Leu	Lys	Lys	Ile	Asp	Val	Leu
	290					295					300				
Asn	Leu	Ser	Ile	Gly	Gly	Pro	Asp	Phe	Met	Asp	His	Pro	Phe	Val	Asp
305					310					315					320
Lys	Val	Trp	Glu	Leu	Thr	Ala	Asn	Asn	Val	Ile	Met	Val	Ser	Ala	Ile
				325					330					335	
Gly	Asn	Asp	Gly	Pro	Leu	Tyr	Gly	Thr	Leu	Asn	Asn	Pro	Ala	Asp	Gln
			340					345					350		
Met	Asp	Val	Ile	Gly	Val	Gly	Gly	Ile	Asp	Phe	Glu	Asp	Asn	Ile	Ala
		355					360					365			
Arg	Phe	Ser	Ser	Arg	Gly	Met	Thr	Thr	Trp	Glu	Leu	Pro	Gly	Gly	Tyr
	370					375					380				
Gly	Arg	Met	Lys	Pro	Asp	Ile	Val	Thr	Tyr	Gly	Ala	Gly	Val	Arg	Gly
385					390					395					400
Ser	Gly	Val	Lys	Gly	Gly	Cys	Arg	Ala	Leu	Ser	Gly	Thr	Ser	Val	Ala
				405					410					415	
Ser	Pro	Val	Val	Ala	Gly	Ala	Val	Thr	Leu	Leu	Val	Ser	Thr	Val	Gln
			420					425					430		
Lys	Arg	Glu	Leu	Val	Asn	Pro	Ala	Ser	Met	Lys	Gln	Ala	Leu	Ile	Ala
		435					440					445			
Ser	Ala	Arg	Arg	Leu	Pro	Gly	Val	Asn	Met	Phe	Glu	Gln	Gly	His	Gly
	450					455					460				
Lys	Leu	Asp	Leu	Leu	Arg	Ala	Tyr	Gln	Ile	Leu	Asn	Ser	Tyr	Lys	Pro
465					470					475					480
Gln	Ala	Ser	Leu	Ser	Pro	Ser	Tyr	Ile	Asp	Leu	Thr	Glu	Cys	Pro	Tyr
				485					490					495	
Met	Trp	Pro	Tyr	Cys	Ser	Gln	Pro	Ile	Tyr	Tyr	Gly	Gly	Met	Pro	Thr
			500					505					510		
Val	Val	Asn	Val	Thr	Ile	Leu	Asn	Gly	Met	Gly	Val	Thr	Gly	Arg	Ile
		515					520					525			
Val	Asp	Lys	Pro	Asp	Trp	Gln	Pro	Tyr	Leu	Pro	Gln	Asn	Gly	Asp	Asn
	530					535					540				
Ile	Glu	Val	Ala	Phe	Ser	Tyr	Ser	Ser	Val	Leu	Trp	Pro	Trp	Ser	Gly
545					550					555					560
Tyr	Leu	Ala	Ile	Ser	Ile	Ser	Val	Thr	Lys	Lys	Ala	Ala	Ser	Trp	Glu
				565					570					575	
Gly	Ile	Ala	Gln	Gly	His	Val	Met	Ile	Thr	Val	Ala	Ser	Pro	Ala	Glu
			580					585					590		

- 82 -

Thr	Glu	Ser	Lys	Asn	Gly	Ala	Glu	Gln	Thr	Ser	Thr	Val	Lys	Leu	Pro	595	600	605	
Ile	Lys	Val	Lys	Ile	Ile	Pro	Thr	Pro	Pro	Arg	Ser	Lys	Arg	Val	Leu	610	615	620	
Trp	Asp	Gln	Tyr	His	Asn	Leu	Arg	Tyr	Pro	Pro	Gly	Tyr	Phe	Pro	Arg	625	630	635	640
Asp	Asn	Leu	Arg	Met	Lys	Asn	Asp	Pro	Leu	Asp	Trp	Asn	Gly	Asp	His	645	650	655	
Ile	His	Thr	Asn	Phe	Arg	Asp	Met	Tyr	Gln	His	Leu	Arg	Ser	Met	Gly	660	665	670	
Tyr	Phe	Val	Glu	Val	Leu	Gly	Ala	Pro	Phe	Thr	Cys	Phe	Asp	Ala	Ser	675	680	685	
Gln	Tyr	Gly	Thr	Leu	Leu	Met	Val	Asp	Ser	Glu	Glu	Glu	Tyr	Phe	Pro	690	695	700	
Glu	Glu	Ile	Ala	Lys	Leu	Arg	Arg	Asp	Val	Asp	Asn	Gly	Leu	Ser	Leu	705	710	715	720
Val	Ile	Phe	Ser	Asp	Trp	Tyr	Asn	Thr	Ser	Val	Met	Arg	Lys	Val	Lys	725	730	735	
Phe	Tyr	Asp	Glu	Asn	Thr	Arg	Gln	Trp	Trp	Met	Pro	Asp	Thr	Gly	Gly	740	745	750	
Ala	Asn	Ile	Pro	Ala	Leu	Asn	Glu	Leu	Leu	Ser	Val	Trp	Asn	Met	Gly	755	760	765	
Phe	Ser	Asp	Gly	Leu	Tyr	Glu	Gly	Glu	Phe	Thr	Leu	Ala	Asn	His	Asp	770	775	780	
Met	Tyr	Tyr	Ala	Ser	Gly	Cys	Ser	Ile	Ala	Lys	Phe	Pro	Glu	Asp	Gly	785	790	795	800
Val	Val	Ile	Thr	Gln	Thr	Phe	Lys	Asp	Gln	Gly	Leu	Glu	Val	Leu	Lys	805	810	815	
Gln	Glu	Thr	Ala	Val	Val	Glu	Asn	Val	Pro	Ile	Leu	Gly	Leu	Tyr	Gln	820	825	830	
Ile	Pro	Ala	Glu	Gly	Gly	Gly	Arg	Ile	Val	Leu	Tyr	Gly	Asp	Ser	Asn	835	840	845	
Cys	Leu	Asp	Asp	Ser	His	Arg	Gln	Lys	Asp	Cys	Phe	Trp	Leu	Leu	Asp	850	855	860	
Ala	Leu	Leu	Gln	Tyr	Thr	Ser	Tyr	Gly	Val	Thr	Pro	Pro	Ser	Leu	Ser	865	870	875	880
His	Ser	Gly	Asn	Arg	Gln	Arg	Pro	Pro	Ser	Gly	Ala	Gly	Ser	Val	Thr	885	890	895	
Pro	Glu	Arg	Met	Glu	Gly	Asn	His	Leu	His	Arg	Tyr	Ser	Lys	Val	Leu	900	905	910	
Glu	Ala	His	Leu	Gly	Asp	Pro	Lys	Pro	Arg	Pro	Leu	Pro	Ala	Cys	Pro				

- 83 -

915		920		925
Arg Leu Ser Trp Ala Lys Pro Gln Pro Leu Asn Glu Thr Ala Pro Ser				
930		935		940
Asn Leu Trp Lys His Gln Lys Leu Leu Ser Ile Asp Leu Asp Lys Val				
945		950		955
Val Leu Pro Asn Phe Arg Ser Asn Arg Pro Gln Val Arg Pro Leu Ser				
		965		970
Pro Gly Glu Ser Gly Ala Trp Asp Ile Pro Gly Gly Ile Met Pro Gly				
		980		985
Arg Tyr Asn Gln Glu Val Gly Gln Thr Ile Pro Val Phe Ala Phe Leu				
		995		1000
				1005
Gly Ala Met Val Val Leu Ala Phe Phe Val Val Gln Ile Asn Lys Ala				
1010		1015		1020
Lys Ser Arg Pro Lys Arg Arg Lys Pro Arg Val Lys Arg Pro Gln Leu				
1025		1030		1035
				1040
Met Gln Gln Val His Pro Pro Lys Thr Pro Ser Val				
		1045		1050

<210> 209

<211> 280

<212> PRT

<213> Homo sapiens

<400> 209

Arg Ala Ile Pro Arg Gln Val Ala Gln Thr Leu Gln Ala Asp Val Leu	
1	5
10	15
Trp Gln Met Gly Tyr Thr Gly Ala Asn Val Arg Val Ala Val Phe Asp	
20	25
30	
Thr Gly Leu Ser Glu Lys His Pro His Phe Lys Asn Val Lys Glu Arg	
35	40
45	
Thr Asn Trp Thr Asn Glu Arg Thr Leu Asp Asp Gly Leu Gly His Gly	
50	55
60	
Thr Phe Val Ala Gly Val Ile Ala Ser Met Arg Glu Cys Gln Gly Phe	
65	70
75	80
Ala Pro Asp Ala Glu Leu His Ile Phe Arg Val Phe Thr Asn Asn Gln	
85	90
95	
Val Ser Tyr Thr Ser Trp Phe Leu Asp Ala Phe Asn Tyr Ala Ile Leu	
100	105
110	
Lys Lys Ile Asp Val Leu Asn Leu Ser Ile Gly Gly Pro Asp Phe Met	
115	120
125	
Asp His Pro Phe Val Asp Lys Val Trp Glu Leu Thr Ala Asn Asn Val	
130	135
140	
Ile Met Val Ser Ala Ile Gly Asn Asp Gly Pro Leu Tyr Gly Thr Leu	

- 84 -

145		150		155		160									
Asn	Asn	Pro	Ala	Asp	Gln	Met	Asp	Val	Ile	Gly	Val	Gly	Gly	Ile	Asp
				165					170					175	
Phe	Glu	Asp	Asn	Ile	Ala	Arg	Phe	Ser	Ser	Arg	Gly	Met	Thr	Thr	Trp
			180					185					190		
Glu	Leu	Pro	Gly	Gly	Tyr	Gly	Arg	Met	Lys	Pro	Asp	Ile	Val	Thr	Tyr
		195					200					205			
Gly	Ala	Gly	Val	Arg	Gly	Ser	Gly	Val	Lys	Gly	Gly	Cys	Arg	Ala	Leu
	210					215						220			
Ser	Gly	Thr	Ser	Val	Ala	Ser	Pro	Val	Val	Ala	Gly	Ala	Val	Thr	Leu
225					230					235					240
Leu	Val	Ser	Thr	Val	Gln	Lys	Arg	Glu	Leu	Val	Asn	Pro	Ala	Ser	Met
				245					250					255	
Lys	Gln	Ala	Leu	Ile	Ala	Ser	Ala	Arg	Arg	Leu	Pro	Gly	Val	Asn	Met
			260					265					270		
Phe	Glu	Gln	Gly	His	Gly	Lys	Leu								
		275					280								

<210> 210

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 210

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 211

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 211

Ala	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 212

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

- 85 -

<400> 212

Gly	Ala	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 213

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 213

Gly	Ser	Ala	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 214

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 214

Gly	Ser	Ile	Ala	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 215

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 215

Gly	Ser	Ile	Ser	Ala	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 216

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 216

Gly	Ser	Ile	Ser	Tyr	Ala	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5					10					15

<210> 217

<211> 15

<212> PRT

<213> Artificial Sequence

- 86 -

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 217

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Ala	Tyr	Ala	Asn	Ala	Met	Ala	Val
1				5				10					15	

<210> 218

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 218

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Ala	Ala	Asn	Ala	Met	Ala	Val
1				5				10					15	

<210> 219

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 219

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Ala	Ala	Met	Ala	Val
1				5				10					15	

<210> 220

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 220

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Ala	Ala	Val
1				5				10					15	

<210> 221

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 221

Gly	Ser	Ile	Ser	Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Ala
1				5				10					15	

- 87 -

<210> 222
 <211> 15
 <212> PRT
 <213> Humicola insolens

<400> 222
 Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln Thr Pro Trp Ala
 1 5 10 15

<210> 223
 <211> 15
 <212> PRT
 <213> Humicola insolens

<400> 223
 Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser
 1 5 10 15

<210> 224
 <211> 276
 <212> PRT
 <213> Humicola insolens

<400> 224
 Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro
 1 5 10 15

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

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
 35 40 45

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
 50 55 60

Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
 65 70 75 80

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
 85 90 95

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
 100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
 115 120 125

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
 130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
 145 150 155 160

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
 165 170 175

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
 180 185 190

- 88 -

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
 195 200 205
 Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
 210 215 220
 Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser
 225 230 235 240
 Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Ser Thr Ser Thr Thr
 245 250 255
 Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
 260 265 270
 Arg Trp Ala Gln
 275

<210> 225
 <211> 18
 <212> PRT
 <213> Thermomyces lanuginosus

<400> 225
 Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys Leu Ile
 1 5 10 15

Val Leu

<210> 226
 <211> 15
 <212> PRT
 <213> Thermomyces lanuginosus

<400> 226
 Ser Ile Glu Asn Trp Ile Gly Asn Leu Asn Phe Asp Leu Lys Glu
 1 5 10 15

<210> 227
 <211> 291
 <212> PRT
 <213> Thermomyces lanuginosus

<400> 227
 Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
 1 5 10 15

Ala Ser Pro Ile Arg Arg Glu Val Ser Gln Asp Leu Phe Asn Gln Phe
 20 25 30

Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn
 35 40 45

Asp Ala Pro Ala Gly Thr Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro
 50 55 60

- 89 -

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

<210> 228

<211> 15

<212> PRT

<213> Streptomyces plicatus

<400> 228

Ile Lys Val Leu Leu Ser Val Leu Gly Asn His Gln Gly Ala Gly
 1 5 10 15

<210> 229

<211> 313

<212> PRT

<213> Streptomyces plicatus

Met 1	Phe	Thr	Pro	Val 5	Arg	Arg	Arg	Val	Arg 10	Thr	Ala	Ala	Leu	Ala 15	Leu
Ser	Ala	Ala	Ala 20	Ala	Leu	Val	Leu	Gly 25	Ser	Thr	Ala	Ala	Ser 30	Gly	Ala
Ser	Ala	Thr 35	Pro	Ser	Pro	Ala	Pro 40	Ala	Pro	Ala	Pro	Ala 45	Pro	Val	Lys
Gln	Gly 50	Pro	Thr	Ser	Val	Ala 55	Tyr	Val	Glu	Val	Asn 60	Asn	Asn	Ser	Met
Leu 65	Asn	Val	Gly	Lys	Tyr 70	Thr	Leu	Ala	Asp	Gly 75	Gly	Gly	Asn	Ala	Phe 80
Asp	Val	Ala	Val	Ile 85	Phe	Ala	Ala	Asn	Ile 90	Asn	Tyr	Asp	Thr	Gly 95	Thr
Lys	Thr	Ala	Tyr 100	Leu	His	Phe	Asn	Glu 105	Asn	Val	Gln	Arg	Val 110	Leu	Asp
Asn	Ala	Val 115	Thr	Gln	Ile	Arg	Pro 120	Leu	Gln	Gln	Gln	Gly 125	Ile	Lys	Val
Leu 130	Leu	Ser	Val	Leu	Gly	Asn 135	His	Gln	Gly	Ala	Gly 140	Phe	Ala	Asn	Phe
Pro 145	Ser	Gln	Gln	Ala	Ala 150	Ser	Ala	Phe	Ala	Lys 155	Gln	Leu	Ser	Asp	Ala 160
Val	Ala	Lys	Tyr	Gly 165	Leu	Asp	Gly	Val	Asp 170	Phe	Asp	Asp	Glu	Tyr 175	Ala
Glu	Tyr	Gly	Asn 180	Asn	Gly	Thr	Ala	Gln 185	Pro	Asn	Asp	Ser	Ser 190	Phe	Val
His	Leu 195	Val	Thr	Ala	Leu	Arg	Ala 200	Asn	Met	Pro	Asp	Lys 205	Ile	Ile	Ser
Leu	Tyr 210	Asn	Ile	Gly	Pro	Ala 215	Ala	Ser	Arg	Leu	Ser 220	Tyr	Gly	Gly	Val
Asp 225	Val	Ser	Asp	Lys	Phe 230	Asp	Tyr	Ala	Trp	Asn 235	Pro	Tyr	Tyr	Gly	Thr 240
Trp	Gln	Val	Pro	Gly 245	Ile	Ala	Leu	Pro	Lys 250	Ala	Gln	Leu	Ser	Pro 255	Ala
Ala	Val	Glu	Ile 260	Gly	Arg	Thr	Ser	Arg 265	Ser	Thr	Val	Ala	Asp 270	Leu	Ala
Arg	Arg	Thr 275	Val	Asp	Glu	Gly	Tyr 280	Gly	Val	Tyr	Leu	Thr 285	Tyr	Asn	Leu
Asp	Gly 290	Gly	Asp	Arg	Thr	Ala 295	Asp	Val	Ser	Ala	Phe 300	Thr	Arg	Glu	Leu
Tyr 305	Gly	Ser	Glu	Ala	Val	Arg	Thr	Pro							

- 91 -

<210> 230
 <211> 15
 <212> PRT
 <213> Bacillus amyloliquefaciens

<400> 230
 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val
 1 5 10 15

<210> 231
 <211> 15
 <212> PRT
 <213> Bacillus amyloliquefaciens

<400> 231
 Asn Gly Ile Glu Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn
 1 5 10 15

<210> 232
 <211> 15
 <212> PRT
 <213> Bacillus lentus

<400> 232
 Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp Thr Gly Ile Ser
 1 5 10 15

<210> 233
 <211> 15
 <212> PRT
 <213> Bacillus lentus

<400> 233
 Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala Ser Gly Ser
 1 5 10 15

<210> 234
 <211> 17
 <212> PRT
 <213> Bacillus lentus

<400> 234
 Gly Ser Ile Ser Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly
 1 5 10 15

Ala

<210> 235
 <211> 15
 <212> PRT
 <213> Bacillus lentus

<400> 235
 Gly Ala Gly Leu Asp Ile Val Ala Pro Gly Val Asn Val Gln Ser

```

1              5              10              15

<210> 236
<211> 272
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Hybrid of
      Bacillus lentus and Bacillus amyloliquefaciens

<400> 236
Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
  1              5              10              15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
      20              25              30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
      35              40              45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
      50              55              60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
  65              70              75              80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
      85              90              95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
      100             105             110

Gly Asn Asn Gly Met His Val Ile Asn Met Ser Leu Gly Gly Ser Gly
      115             120             125

Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala Ser Gly Val
      130             135             140

Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Ser
  145             150             155             160

Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala
      165             170             175

Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu
      180             185             190

Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly
      195             200             205

Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val
      210             215             220

Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn
  225             230             235             240

Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys Leu Gly Asp
      245             250             255

```

- 93 -

Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
 260 265 270

<210> 237
 <211> 15
 <212> PRT
 <213> Bacillus lentis subtilisin

<400> 237
 Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro
 1 5 10 15

<210> 238
 <211> 18
 <212> PRT
 <213> Bacillus lentis subtilisin

<400> 238
 Leu Glu Trp Ala Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu
 1 5 10 15
 Gly Ser

<210> 239
 <211> 15
 <212> PRT
 <213> Bacillus amyloliquefaciens subtilisin

<400> 239
 Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro
 1 5 10 15

<210> 240
 <211> 17
 <212> PRT
 <213> Bacillus amyloliquefaciens subtilisin

<400> 240
 Ile Glu Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu
 1 5 10 15
 Gly

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method to determine the relative allergenic potential of an engineered protein comprising the steps of,

a) immunizing a first transgenic mouse with a protein of interest and immunizing a second transgenic mouse with an engineered protein wherein said engineered protein is a variant of said protein of interest and said protein of interest includes a T-cell epitope wherein the variant differs from the protein of interest by having an altered T cell epitope;

b) collecting serum of said first and said second immunized transgenic mice;

c) measuring the serum for antigen specific immunoglobulins; and

d) comparing the immunogenic response of said variant and said protein of interest thereby determining the relative allergenic potential of the protein of interest and the engineered protein, wherein the first transgenic mouse and second transgenic mouse are HLA D3R/DQ2 and wherein the variant and the protein of interest produce a different immunogenic response in said transgenic mice.

2. The method according to claim 1, wherein said protein of interest is an enzyme.

3. The method according to claim 2, wherein said enzyme is a protease.

4. The method according to claim 1, wherein the antigen specific immunoglobulin is IgG.

5. The method according to claim 1, wherein the HLA DR3/DQ2 transgenic mice have been backcrossed with mice lacking the expression of endogenous I-A class II molecules.

6. The method according to claim 1, wherein said T-cell epitope is altered with amino acid substitutions.
7. The method according to claim 1, wherein said T-cell epitope is altered by having a terminal portion of said protein of interest which includes said T-cell epitope replaced with a corresponding terminal portion of a homolog of said protein of interest wherein said homolog comprises a T-cell epitope that is different to said replaced T-cell epitope.
8. The method according to claim 1, wherein said immunogenic response produced by the variant is less than the immunogenic response produced by the protein of interest.
9. The method according to claim 1, wherein said immunogenic response produced by the variant is more than the immunogenic response produced by the protein of interest.
10. The method of claim 1, wherein said immunogenic response is predictive of the allergenic response in humans.
11. The method according to claim 10, wherein said protein of interest is a protease.

1 / 28

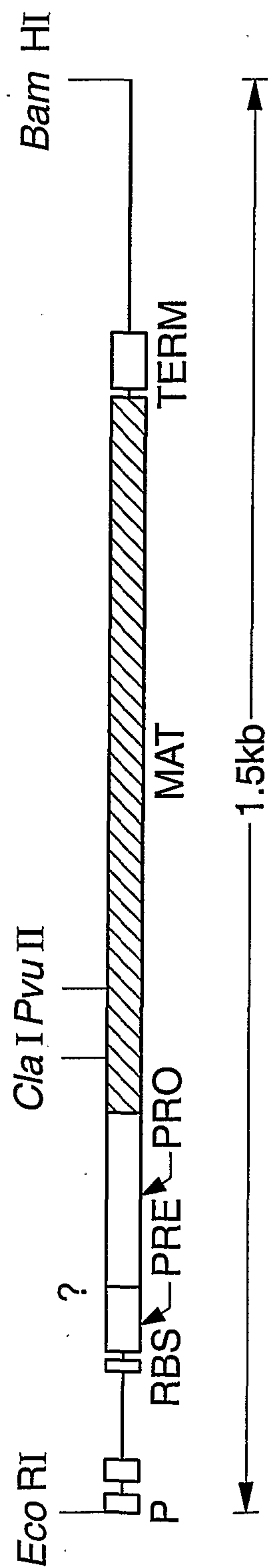
**FIG. 1A**

FIG. 1B - 1

50	Pro	Asn	Phe	Gln	Asp	Asp	Asn	Asn	Ser	His	Gly	Thr	His	Val	Ala											
549	GCA	GGC	GGA	GCC	AGC	ATG	GTT	CCT	CCT	TCT	GAA	ACA	ACA	AAT	CCT	TTC	CAA	GAC	AAC	TCT	CAC	GGA	ACT	CAC	GTT	GCC
70	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Gly	Val	Val	Ala	Pro	Ser	Ala	Ser	Ala	90	Tyr	Ala	Val	Lys	
624	GGC	ACA	GTT	GCG	GCT	CTT	AAT	AAC	TCA	ATC	GGT	GTA	TTC	GGC	GTT	GCG	CCA	AGC	GCA	TCA	CTT	TAC	GCT	GTA	AAA	
100	Asp	Ala	Ala	Asp	Gly	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu	Trp	Ala	Ala	110	Ala	Asn	Met		
699	GTT	CTC	GGT	GCT	GAC	GGT	TCC	GGC	CAA	TAC	AGC	TGG	ATC	ATT	AAC	GGA	ATC	GAG	TGG	GCG	ATC	GCA	AAC	AAT	ATG	
120	Val	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Val	Val	140	Lys	Ala	Val	Ala	
774	GAC	GTT	ATT	AAC	ATG	AGC	CTC	GGC	GCA	GCC	GGA	CCT	TCT	TCT	GCT	GCT	TTA	AAA	GCG	GCA	GTT	GAT	AAA	GCC	GTT	GCA
150	Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Gly	Glu	Gly	Thr	Ser	Gly	Ser	Ser	Thr	160	Val	Gly	Tyr	Pro	Gly
849	TCC	GGC	GTC	GTA	GTC	GTT	GCG	GCA	GCC	GGT	AAC	AAC	GAA	GGC	ACT	TCC	GGC	AGC	TCA	AGC	ACA	GTG	GGC	TAC	CCT	GGT
170	Lys	Tyr	Pro	Ser	Val	Ile	Ala	Val	Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	190	Ser	Ser	Val	Gly	Pro
924	AAA	TAC	CCT	TCT	TCT	GTC	ATT	GCA	GTA	GGC	GCT	GTT	GAC	AGC	AGC	AAC	CAA	AGA	GCA	TCT	TTC	TCA	AGC	GTA	GGA	CCT
200	Glu	Leu	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr	Leu	Pro	Gly	Asn	Lys	Tyr	210	Gly	Ala	Tyr	Asn	Gly
999	GAG	CTT	GAT	GTC	ATG	GCA	CCT	GGC	GTA	TCT	ATC	CAA	AGC	ACG	CTT	CCT	GGA	AAC	AAA	TAC	GGG	GCG	TAC	AAC	GGT	
220	Thr	Ser	Met	Ala	Ser	Pro	His	Val	Val	Ala	Gly	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	240	Asn	Trp	Thr	Asn	Thr
1074	ACG	TCA	ATG	GCA	TCT	CCG	CAC	GTT	GCC	GCT	GCG	GCT	GCT	TTG	ATT	CTT	TCT	AAG	CAC	CCG	AAC	TGG	ACA	AAC	ACT	

3 / 28

FIG. 1B - 2

1416 CTCCCGGTTCCGGTCAGCTCAATGCCGTACGGTCGGCGGTTTCCTGATACCGGAGACGGCATTCCGTAATCGGATC

FIG. 1B - 3

FIG. 1B

FIG. 1B-1

FIG. 1B-2

FIG. 1B - 3

5 / 28

CONSERVED RESIDUES IN SUBTILISINS FROM
BACILLUS AMYLOLIQUEFACIENS

1	10	20
A Q S V P . G	A P A . H . .	G
21	30	40
. T G S . V K V A V . D . G	H P	
41	50	60
D L . . . G G A S . V P	Q D	
61	70	80
. N . H G T H V A G T . A A L N N S I G		
81	90	100
V L G V A P S A . L Y A V K V L G A . G		
101	110	120
S G . . S . L . . G . E W A . N		
121	130	140
V . N . S L G . P S . S	A . .	
141	150	160
. G V . V V A A . G N . G . . .		
161	170	180
. Y P . . Y A V G A .		
181	190	200
D . . N . . A S F S . . G . . L D . . A		
201	210	220
P G V . . Q S T . P G . . Y . . . N G T		
221	230	240
S M A . P H V A G A A A L . . . K . . .		
241	250	260
W . . . Q . R . . L . N T . . . L G . .		
261	270	
. . Y G . G L . N . . A A . .		

FIG. 2

6 / 28

COMPARISON OF SUBTILISIN SEQUENCES FROM:

*B.amyloliquefaciens**B.subtilis**B.licheniformis**B.lentus***FIG._3A**

01	A	Q	S	V	P	Y	G	V	S	Q	I	K	A	P	A	L	H	S	Q	G	Y	T	G	S	N	V	K	V	A	V	I	D	S	G	I	D	S	S	H	P	
	A	Q	S	V	P	Y	G	I	S	Q	I	K	A	P	A	L	H	S	Q	G	Y	T	G	S	N	V	K	V	A	V	I	D	S	G	I	D	S	S	H	P	
	A	Q	T	V	P	Y	G	I	P	L	I	K	A	D	K	V	Q	A	Q	G	F	K	G	A	N	V	K	V	A	V	L	D	T	G	I	Q	A	S	H	P	
	A	Q	S	V	P	W	G	I	S	R	V	Q	A	P	A	A	H	N	R	G	L	T	G	S	G	V	K	V	A	V	L	D	T	G	I	S	T	*	H	P	
41	D	L	K	V	A	G	G	A	S	M	V	P	S	E	T	N	P	F	Q	D	N	N	S	H	G	T	H	V	A	G	T	V	A	A	L	N	N	S	I	G	
	D	L	N	V	R	G	G	A	S	F	V	P	S	E	T	N	P	Y	Q	D	G	N	S	H	G	T	H	V	A	G	T	V	A	A	L	N	N	S	I	G	
	D	L	N	V	V	G	G	A	S	F	V	P	A	G	E	A	Y	*	T	D	G	N	S	H	G	T	H	V	A	G	T	V	A	A	L	N	N	S	I	G	
	D	L	N	I	R	G	G	A	S	F	V	P	G	E	*	P	S	T	Q	D	G	N	G	H	G	T	H	V	A	G	T	V	A	A	L	N	N	S	I	G	
81	V	L	G	V	A	P	S	A	S	L	Y	A	V	K	V	L	G	A	D	G	S	G	Q	Y	S	W	I	I	N	G	I	E	W	A	I	A	N	N	M	D	
	V	L	G	V	S	P	S	A	S	L	Y	A	V	K	V	L	D	S	T	G	S	G	Q	Y	S	W	I	I	N	G	I	E	W	A	I	A	N	N	M	D	
	V	L	G	V	A	P	S	V	S	L	Y	A	V	K	V	L	N	S	S	G	S	G	S	Y	S	G	I	I	V	S	G	I	E	W	A	I	A	N	N	M	D
	V	L	G	V	A	P	S	A	E	L	Y	A	V	K	V	L	G	A	S	G	S	G	S	V	S	I	A	Q	G	L	E	W	A	I	A	N	N	M	D		
121	V	I	N	M	S	L	G	G	P	S	G	S	A	A	L	K	A	A	V	D	K	A	V	A	S	G	V	V	V	V	A	A	A	G	N	E	G	T	S	G	
	V	I	N	M	S	L	G	G	P	T	G	S	T	A	L	K	T	V	V	D	K	A	V	A	S	G	I	V	V	V	A	A	A	G	N	E	G	T	S	G	
	V	I	N	M	S	L	G	G	A	S	G	S	T	A	M	K	Q	A	V	D	N	A	Y	A	R	G	V	V	V	V	A	A	A	G	N	E	G	T	S	G	
	V	A	N	L	S	L	G	S	P	S	P	S	A	T	L	E	Q	A	V	N	S	A	T	S	R	G	V	L	V	V	A	A	A	G	N	E	G	T	S	G	

161 S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
S S T S T V G Y P P A K Y P S T I A V G A V N S S N Q R A S F S S A G S E L D V M A
S T N T I G Y P A K Y P S V I A V G A V D S S N R R A S F S S V G A E L D V M A
* * * I S Y P A R Y A N A M A V G A T D Q N N R A S F S Q Y G A G L D I V A

201 P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A L I L S K H P N
P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A L I L S K H P T
P G A G V Y S T Y P T N T Y A T L N G T S M A S P H V A G A A L I L S K H P N
P G V N V Q S T Y P G S T Y A S L N G T S M A S P H V A G A A L I L S K N P S

241 W T N T Q V R S S L E N T T K L G D S F Y Y G K G L I N V Q A A A Q
W T N A Q V R D R L E S T A T Y L G N S F Y Y G K G L I N V Q A A A Q
L S A S Q V R N R L S S T A T Y L G S S F Y Y G K G L I N V E A A A Q
W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R

FIG._3B

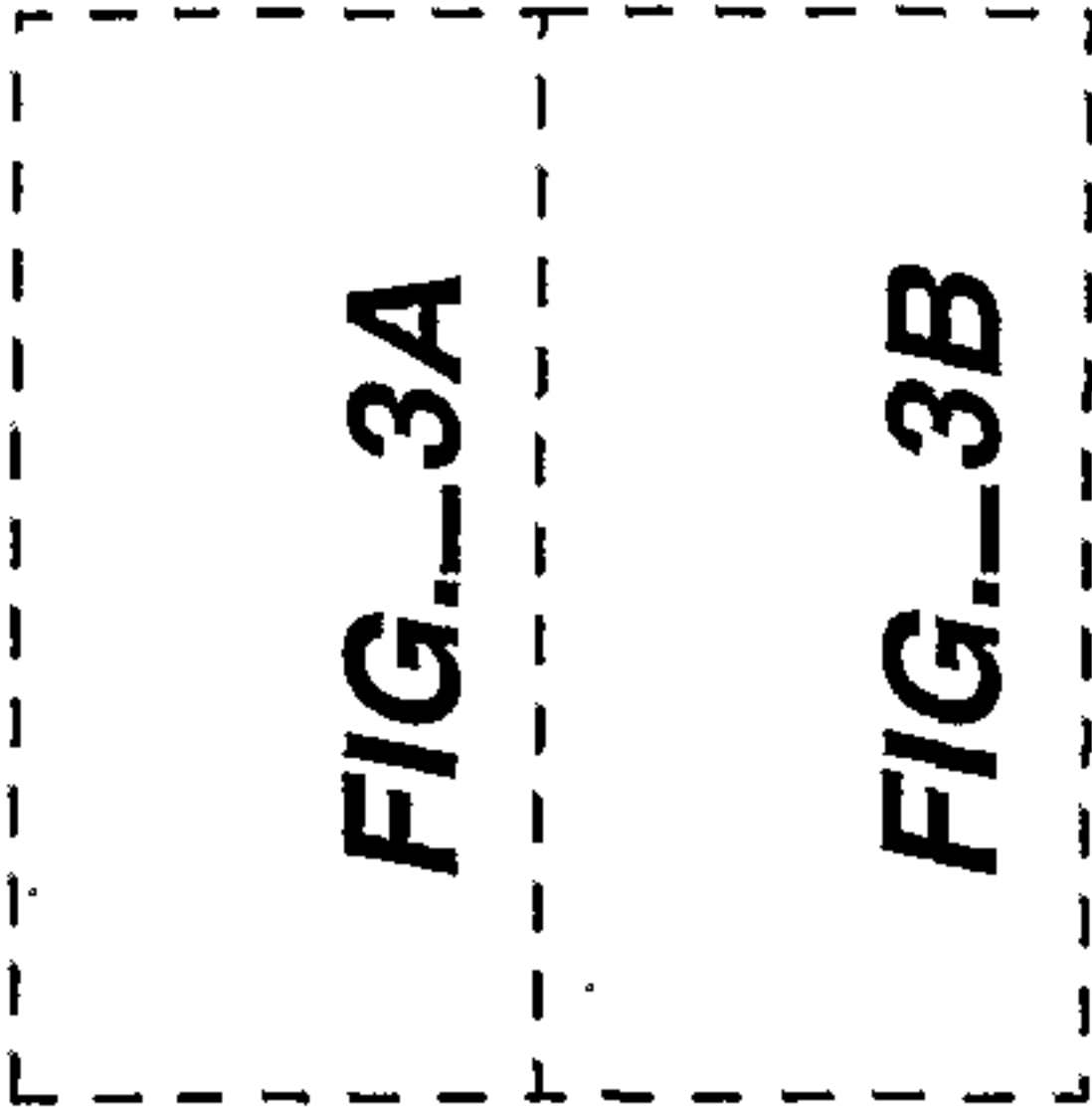


FIG._3

8 / 28

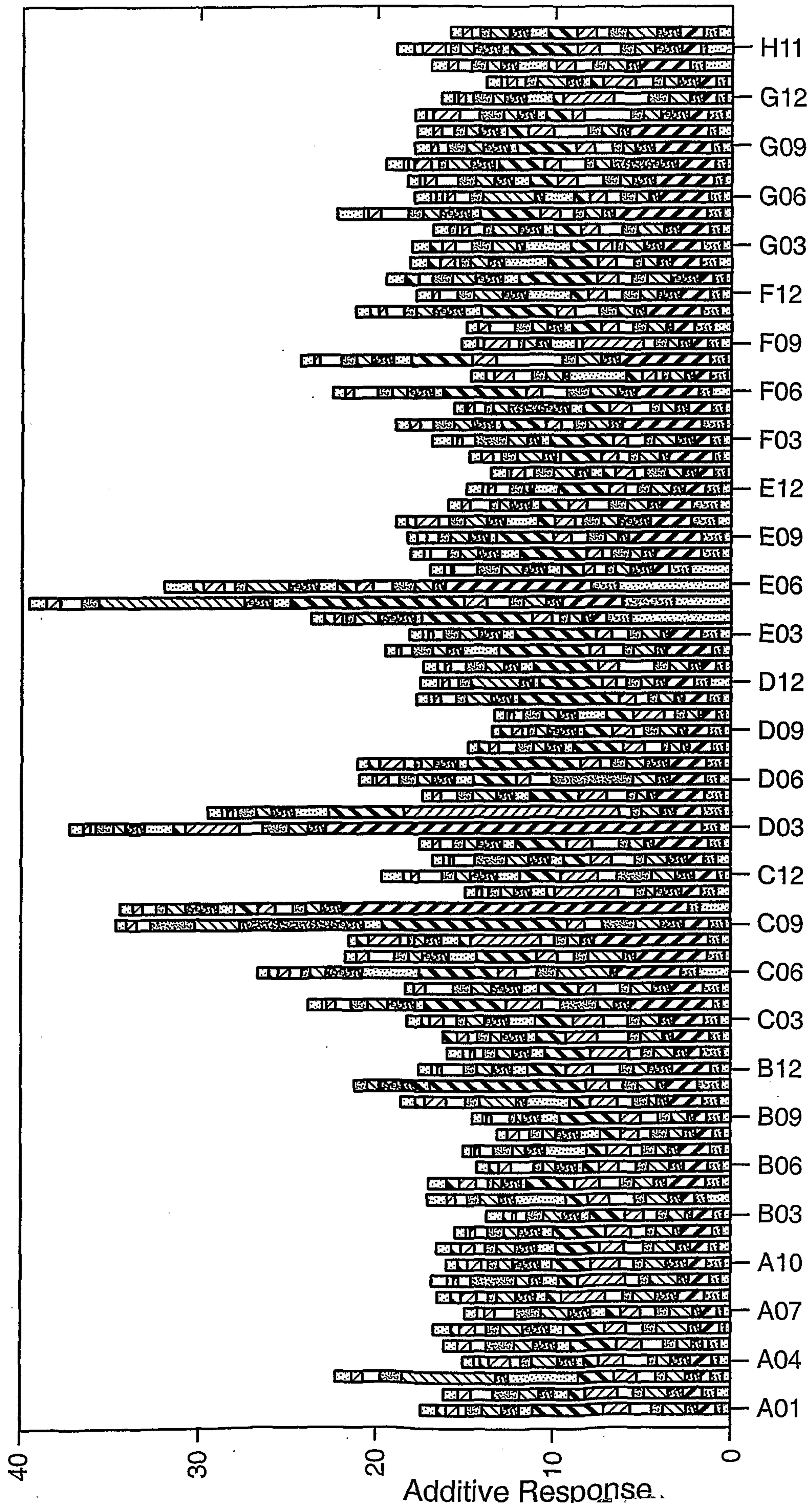


FIG.-4

9 / 28

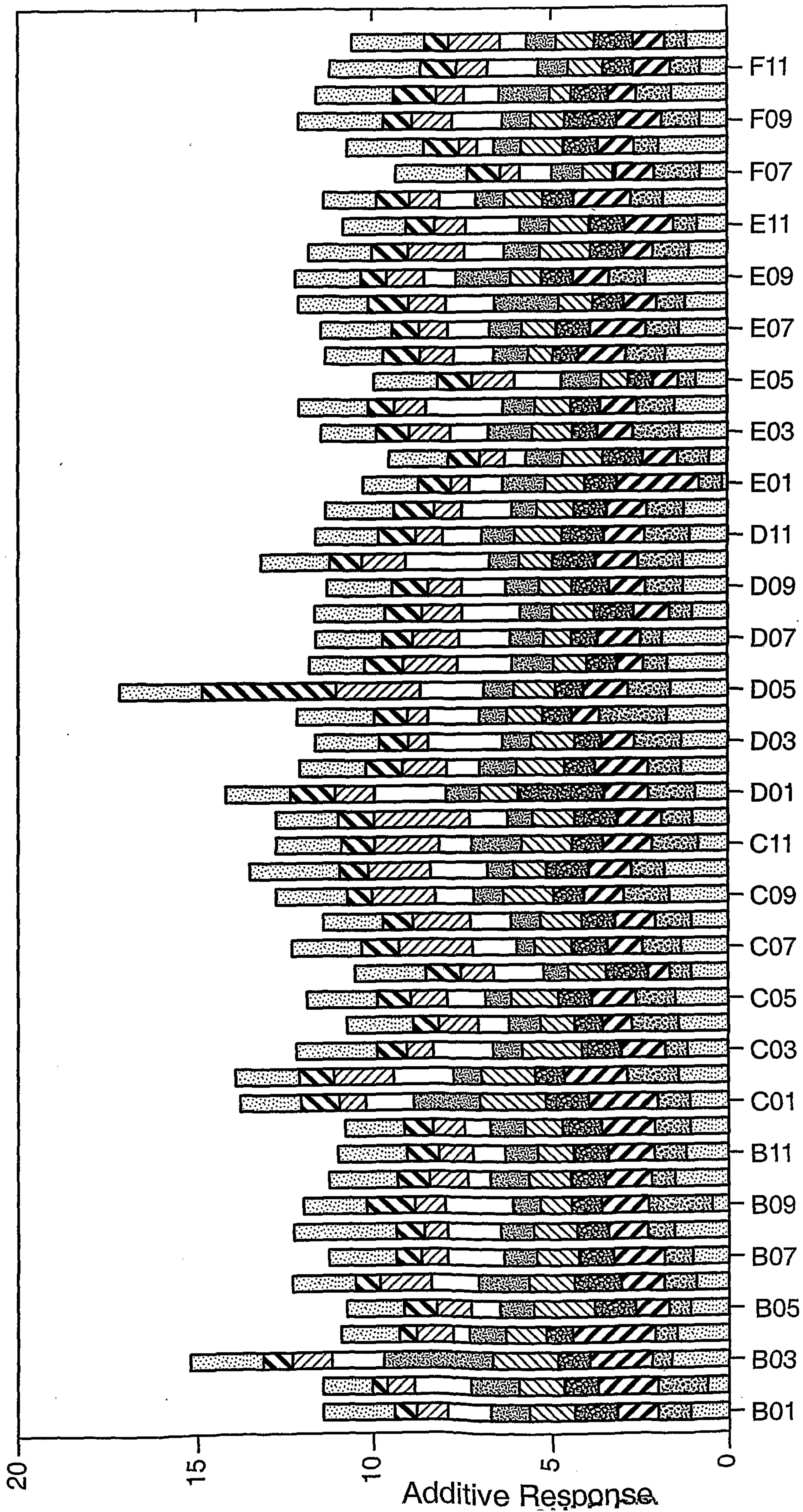


FIG.-5

10 / 28

1	A12	IKDFHVYFRESRDAG	49	E12	SATSRGVLVVAASGN
2	A11	LEQAVNSATSRGVLV	50	E11	SRGVLVVAASGNSGA
3	A10	AQSVPWGISRVQAPA	51	E10	VLVVAASGNSGAGSI
4	A9	VPWGISRVQAPAAHN	52	E9	VAASGNSGAGSISYP
5	A8	GISRVQAPAAHNRGL	53	E8	SGNSGAGSISYPARY
6	A7	RVQAPAAHNRGLTGS	54	E7	SGAGSISYPARYANA
7	A6	APAAHNRGLTGSGVK	55	E6	GSISYPARYANAMAV
8	A5	AHNRGLTGSGVKVAV	56	E5	SYPARYANAMAVGAT
9	A4	RGLTGSGVKVAVLDT	57	E4	ARYANAMAVGATDQN
10	A3	TGSGVKVAVLDTGIS	58	E3	ANAMAVGATDQNNNR
11	A2	GVKVAVLDTGISTHP	59	E2	MAVGATDQNNNRASF
12	A1	VAVLDTGISTHPDLN	60	E1	GATDQNNNRASFQY
13	B12	LDTGISTHPDLNIRG	61	F12	DQNNNRASFQYGAG
14	B11	GISTHPDLNIRGGAS	62	F11	NNRASFSQYGAGLDI
15	B10	THPDLNIRGGASFVP	63	F10	ASFQYGAGLDIVAP
16	B9	DLNIRGGASFVPGEF	64	F9	SQYGAGLDIVAPGVN
17	B8	IRGGASFVPGEFSTQ	65	F8	GAGLDIVAPGVNVQS
18	B7	GASFVPGEFSTQDGN	66	F7	LDIVAPGVNVQSTYP
19	B6	FVPGEFSTQDGNHGH	67	F6	VAPGVNVQSTYPGST
20	B5	GEPSTQDGNHGHGTHV	68	F5	GVNVQSTYPGSTYAS
21	B4	STQDGNHGHGTHVAGT	69	F4	VQSTYPGSTYASLNG
22	B3	DGNHGHGTHVAGTIAA	70	F3	TYPGSTYASLNGTSM
23	B2	GHGTHVAGTIAALNN	71	F2	GSTYASLNGTSMATP
24	B1	THVAGTIAALNNSIG	72	F1	YASLNGTSMATPHVA
25	C12	AGTIAALNNSIGVLG	73	G12	LNGTSMATPHVAGAA
26	C11	IAALNNSIGVLGVAP	74	G11	TSMATPHVAGAAALV
27	C10	LNNSIGVLGVAPSAE	75	G10	ATPHVAGAAALVKQK
28	C9	SIGVLGVAPSAELYA	76	G9	HVAGAAALVKQKNPS
29	C8	VLGVAPSAELYAVKV	77	G8	GAAALVKQKNPSWSN
30	C7	VAPSAELYAVKVLGA	78	G7	ALVKQKNPSWSNVQI
31	C6	SAELYAVKVLGASGS	79	G6	KQKNPSWSNVQIRNH
32	C5	LYAVKVLGASGSGSV	80	G5	NPSWSNVQIRNHLKN
33	C4	VKVLGASGSGSVSSI	81	G4	WSNVQIRNHLKNTAT
34	C3	LGASGSGSVSSIAQG	82	G3	VQIRNHLKNTATSLG
35	C2	SGSGSVSSIAQGLEW	83	G2	RNHLKNTATSLGSTN
36	C1	GSVSSIAQGLEWAGN	84	G1	LKNTATSLGSTNLYG
37	D12	SSIAQGLEWAGNNGM	85	H12	TATSLGSTNLYGSGL
38	D11	AQGLEWAGNNGMHVA	86	H11	SLGSTNLYGSGLVNA
39	D10	LEWAGNNGMHVANLS	87	H10	STNLYGSGLVNAEAA
40	D9	AGNNGMHVANLSLGS	88	H9	NLYGSGLVNAEAATR
41	D8	NGMHVANLSLGSPPSP			
42	D7	HVANLSLGSPPSPSAT			
43	D6	NLSLGSPPSPSATLEQ			
44	D5	LGSPSPSATLEQAVN			
45	D4	PSPSATLEQAVNSAT			
46	D3	SATLEQAVNSATSRG			
47	D2	LEQAVNSATSRGVLV			
48	D1	AVNSATSRGVLVVA			

FIG. 6A

11 / 28

1	A12	IKDFHVYFRESRDAG	49	E12	KKIDVLNLSIGGPDF
2	A11	DAELHIFRVFTNNQV	50	E11	DVLNLSIGGPDFMDH
3	A10	PLRRASLSLGS GFHW	51	E10	NLSIGGPDFMDHPFV
4	A9	RASLSLGS GFWHATG	52	E9	IGGPDFMDHPFVDKV
5	A8	LSLGS GFWHATGRHS	53	E8	PDFMDHPFVDK VWEL
6	A7	GSG FWHATGRHSSRR	54	E7	MDHPFVDK VWELTAN
7	A6	FWHATGRHSSRLLR	55	E6	PFVDK VWELTANNVI
8	A5	ATGRHSSRLLRAIP	56	E5	DK VWELTANNVIMVS
9	A4	RHSSRLLRAIPRQV	57	E4	WELTANNVIMVSAIG
10	A3	SRLLRAIPRQVAQT	58	E3	TANNVIMVSAIGNDG
11	A2	LLRAIPRQVAQTLQA	59	E2	NVIMVSAIGNDGPLY
12	A1	AIPRQVAQTLQADVL	60	E1	MVSAIGNDGPLYGTI
13	B12	RQVAQTLQADVLWQM	61	F12	AIGNDGPLYGTLNNP
14	B11	AQTLQADVLWQMGYT	62	F11	NDGPLYGTLNNPADQ
15	B10	LQADVLWQMGYTGAN	63	F10	PLYGTLNNPADQMDV
16	B9	DVLWQMGYTGANVRV	64	F9	GTLNNPADQMDVIGV
17	B8	WQMGYTGANVRVAVF	65	F8	NNPADQMDVIGVGGI
18	B7	GYTGANVRVAVFDTG	66	F7	ADQMDVIGVGGIDFE
19	B6	GANVRVAVFDTGLSE	67	F6	MDVIGVGGIDFEDNI
20	B5	VRVAVFDTGLSEKHP	68	F5	IGVGGIDFEDNIARF
21	B4	AVFDTGLSEKHPHFK	69	F4	GGIDFEDNIARFSSR
22	B3	DTGLSEKHPHFKNVK	70	F3	DFEDNIARFSSRGMT
23	B2	LSEKHPHFKNVKERT	71	F2	DNIARFSSRGMTTWE
24	B1	KHPHFKNVKERTNWT	72	F1	ARFSSRGMTTWELPG
25	C12	HFKNVKERTNWTNER	73	G12	SSRGMTTWELPGGYG
26	C11	NVKERTNWTNERTLD	74	G11	GMTTWELPGGYGRMK
27	C10	ERTNWTNERTLDDGL	75	G10	TWELPGGYGRMKPDI
28	C9	NWTNERTLDDGLGHG	76	G9	LPGGYGRMKPDIVTY
29	C8	NERTLDDGLGHGTFV	77	G8	GYGRMKPDIVTYGAG
30	C7	TLDDGLGHGTFVAGV	78	G7	RMKPDIVTYGAGVRG
31	C6	DGLGHGTFVAGVIAS	79	G6	PDIVTYGAGVRGSGV
32	C5	GHGTFVAGVIASMRE	80	G5	VTYGAGVRGSGVKGG
33	C4	TFVAGVIASMRECQG	81	G4	GAGVRGSGVKGGCRA
34	C3	AGVIASMRECQGFAP	82	G3	VRGSGVKGGCRALSG
35	C2	IASMRECQGFAPDAE	83	G2	SGVKGGCRALSGTSV
36	C1	MRECQGFAPDAELHI	84	G1	KGGCRALSGTSVASP
37	D12	CQGFAPDAELHIFRV	85	H12	CRALSGTSVASPVVA
38	D11	FAPDAELHIFRVFTN	86	H11	LSGTSVASPVVAGAV
39	D10	DAELHIFRVFTNNQV	87	H10	TSVASPVVAGAVTLL
40	D9	LHIFRVFTNNQVSYT	88	H9	ASPVVAGAVTLLVST
41	D8	FRVFTNNQVSYTSWF	89	H8	VVAGAVTLLVSTVQK
42	D7	FTNNQVSYTSWFLDA	90	H7	GAVTLLVSTVQKREL
43	D6	NQVSYTSWFLDAFNY	91	H6	TLLVSTVQKRELVNP
44	D5	SYTSWFLDAFNYAIL	92	H5	VSTVQKRELVNPASM
45	D4	SWFLDAFNYAILKKI	93	H4	VQKRELVNPASMKQA
46	D3	LDAFNYAILKKIDVL	94	H3	RELVNPASMKQALIA
47	D2	FNYAILKKIDVLNLS	95	H2	VNPASMKQALIASAR
48	D1	AILKKIDVLNLSIGG	96	H1	ASMKQALIASARRLP

FIG. 6B

SUBSTITUTE SHEET (RULE 26)

12 / 28

97	I12	IKDFHVYFRESRDAG
98	I11	DAELHIFRVFTNNQV
99	I10	KQALIASARRLPGVN
100	I9	LIASARRLPGVNMFE
101	I8	SARRLPGVNMFEQGH
102	I7	RLPGVNMFEQGHGKL
103	I6	GVNMFEQGHGKLDLL
104	I5	MFEQGHGKLDLLRAY
105	I4	QGHGKLDLLRAYQIL
106	I3	GKLDLLRAYQILNSY
107	I2	DLLRAYQILNSYKPQ
108	I1	RAYQILNSYKPQASL
109	J12	QILNSYKPQASLSPS
110	J11	NSYKPQASLSPSYID
111	J10	KPQASLSPSYIDLTE
112	J9	ASLSPSYIDLTECPY
113	J8	SPSYIDLTECPYMWP
114	J7	YIDLTECPYMWPYCS
115	J6	LTECPYMWPYCSQPI
116	J5	CPYMWPYCSQPIYYG

FIG. 6C

MKLVNIWLLLLVLLCGKKHLGDRLEKKSFEKAPCGCSHLTLKVEFSSTVVEYEVIVAFNGYFT
AKARNSFISSALKSSEVDNWRIIPRNNPSSDYPDSDFEVIQIKEKQKAGLLTLEDHPNIKRVTPQR
KVFRSLKYAESDPTVPCNETRWSQKWQSSRPLRRASLSLGSGFWHATGRHSSRRLLRAIPRQVAQ
TLQADVLRQMGYTGANVRVAVFDTGLSEKHPHFKNVKERTNWTNERTLDDGLGHGTFVAGVIASM
RECQGFAPDAELHIFRVFTNNQVSYTSWFLDAFNYAIIKKIDVLNLSIGGPDFMDHPFVDKVVWEL
TANNVIMVSAIGNDGPLYGTINNPAQMDVIGVGIDFEDNIARFSSRGMTTWELPGGYGRMKPD
IVTYGAGVRGSGVKGCCRALSGTSVASPVVAGAVTLLVSTVQKRELVPASMKQALIASARRLPG
VNMFEQGHGKLLRAYQILNSYKPQASLSPSYIDLTECPYMWPYCSQPIYYGGMPTVVNVVTILN
GMGVTGRIVDKPDWQPYLPQNGDNI EVAFSYSSVLWPWSGYLAISISVTKKAASWEGIAQGHVMI
TVASPAETESKNGAEQTSVKLPKVKIIPTPPRSKRVLWDQYHNLRYPGYPFPRDNLRMKNDPL
DWNGDHIHTNFRDMYQHLSMGYFVEVLGAPFTCFDASQYGTLLMVDSEEEYFP EEIAKLRRDVD
NGLSLVIFSDWYNTSVMRKVKFYDENTRQWMPDTGGANIPALNELLSVWNMGFSDGLYEGETTL
ANHDMYASGCSIAKFPEDGVVITQTFKDQGLEVLKQETA VVENVPILGLYQIPAEGGGRIVLYG
DSNCLDDSHRQKDCFWLLDALLQYTSYGVTPPSLSHSGNRQRPSPGAGSVTPERMENHLHRYSK
VLEAHLGDPKPRPLPACPRLSWAKPQPLNETAPSNLWKHQKLLSIDLDKVVLPNFRSNRPQVRPL
SPGESGAWDIPGGIMPGRYNQEVGQTI PVFAFLGAMVVLAFFVVQINKAKSRPKRRKPRVKRPQL
MQQVHPPKTPSV

FIG.- 7

	260	270	280	290	
BPN'	SKHPNWTNTQ---	VRSSLENTT	TKLGDSFY	YGKGLIN	VQAAAQ
SAVINASE	QKNPSWSNVQ---	IRNHLKNTA	TSLGSTNL	YGSGLVN	AEAAATR
S2HSBT	STVQKREL	VNPASMKQ	ALIASARR	LPGVNMF	EQG----HGKL

15 / 28

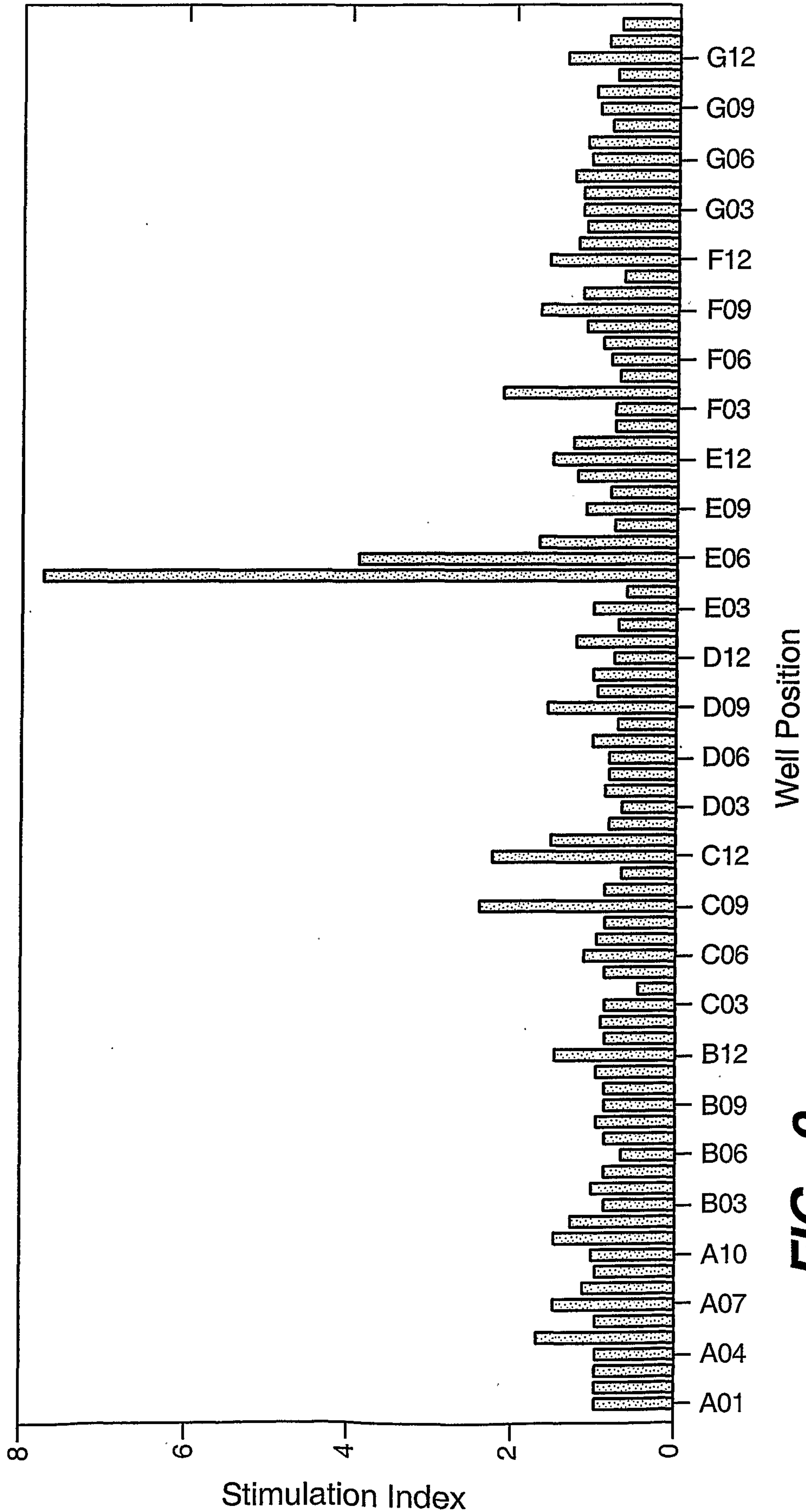
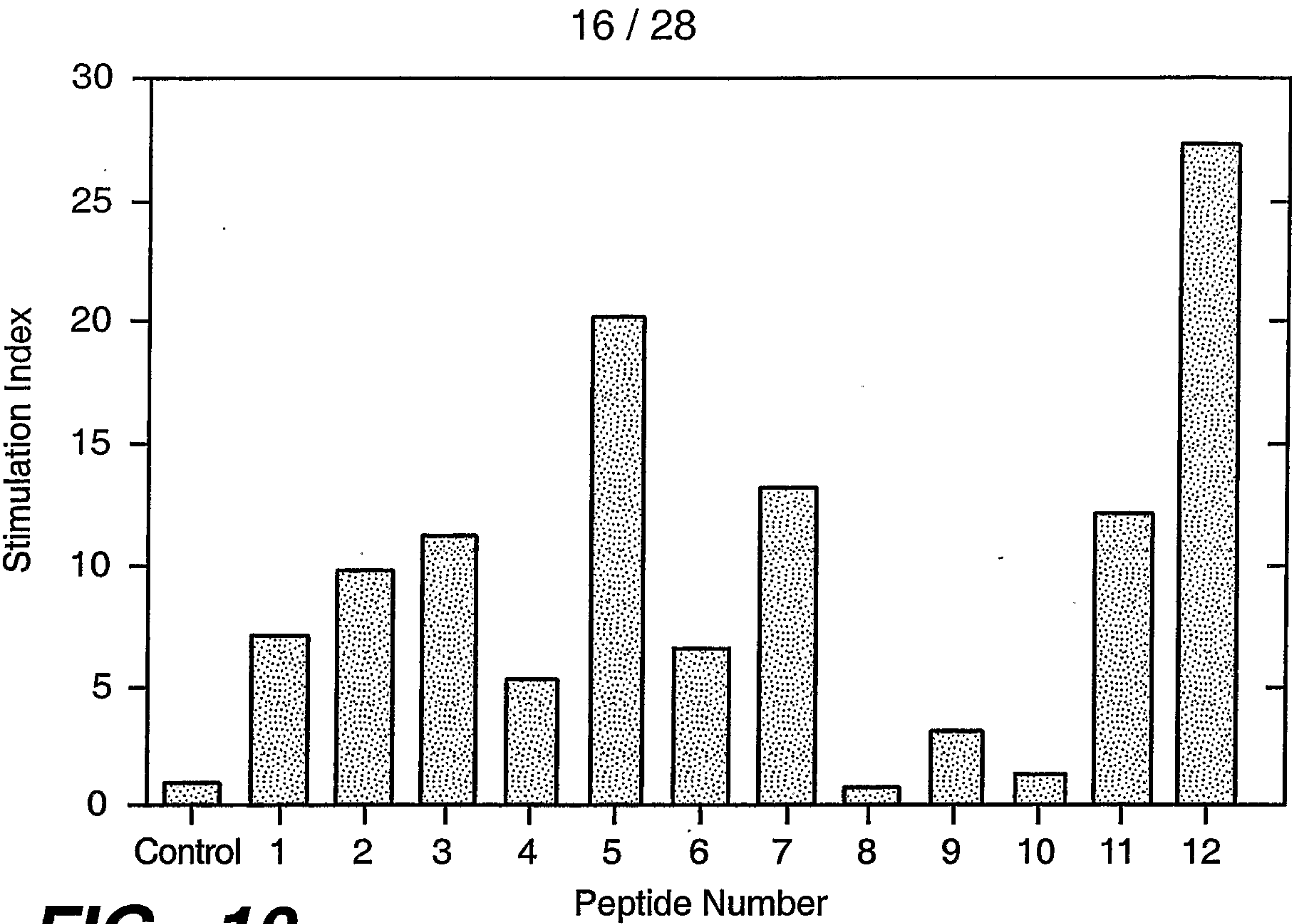


FIG. 9



PEPTIDE NUMBER	SEQUENCE
1 (Unmodified Sequence)	GSISYPARYANAMAV
2	A SISYPARYANAMAV
3	G A ISYPARYANAMAV
4	GS A SYPARYANAMAV
5	GS I A YPARYANAMAV
6	GSIS A PARYANAMAV
7	GSISY A ARYANAMAV
8	GSISYP A AYANAMAV
9	GSISYPAR A ANAMAV
10	GSISYPARY A AMAV
11	GSISYPARYAN A AV
12	GSISYPARYANAM A A

FIG._11

17 / 28

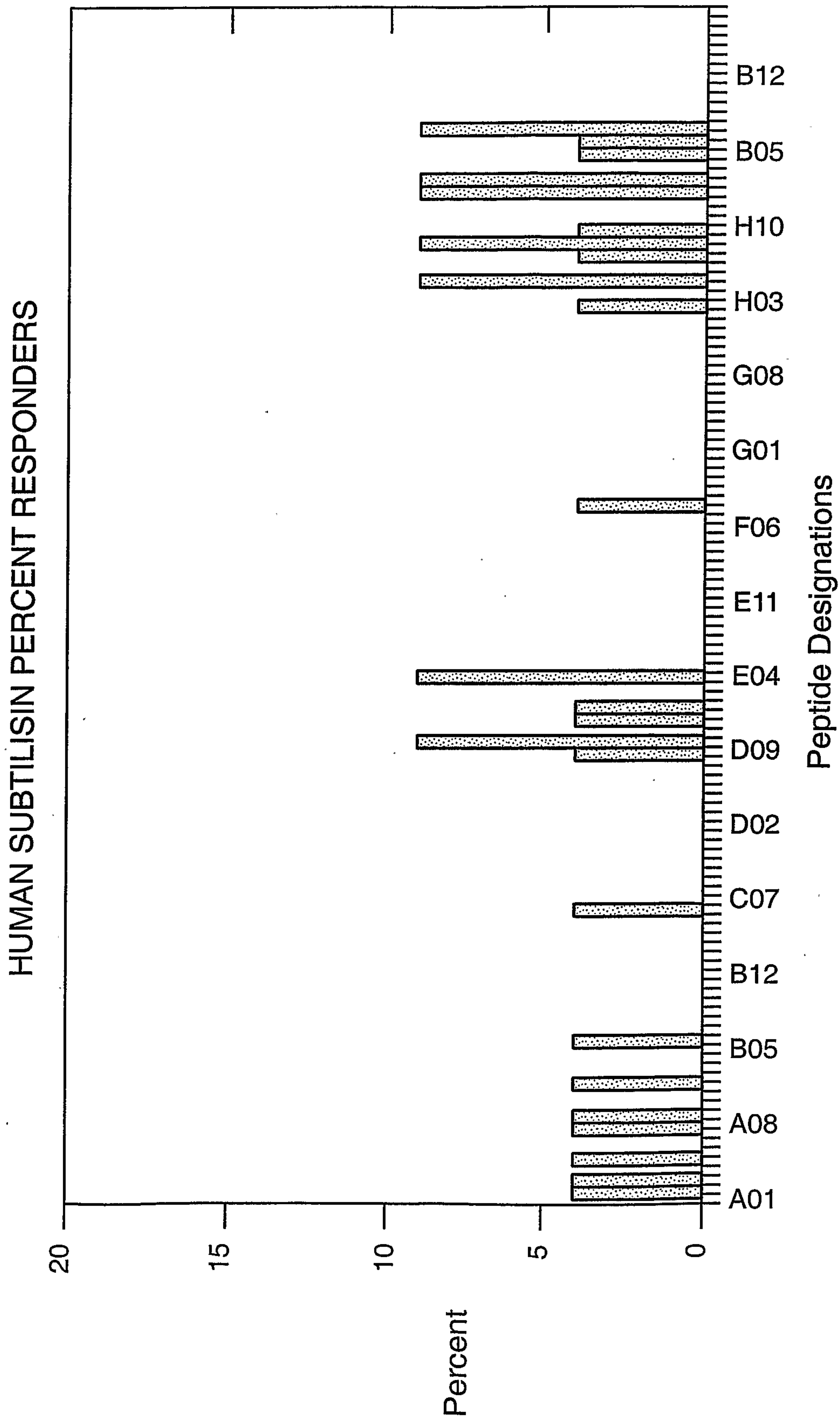
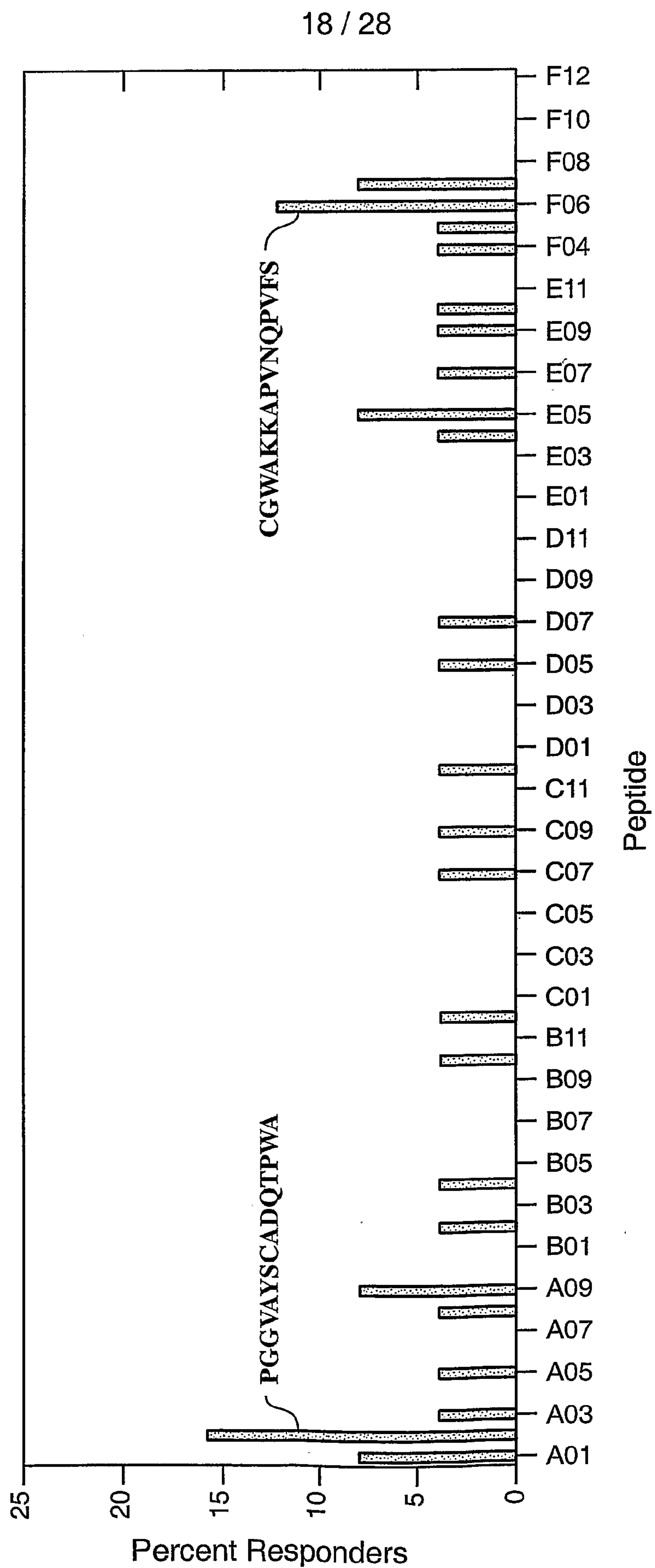


FIG. 12

**FIG. 13A**

19 / 28

1	2	3	4	5
1234567890	1234567890	1234567890	1234567890	1234567890
MRSSPLLPSA	VVAALPVLAL	AADGRSTRYW	DCCKPSCGWA	<u>KKAPVNQPVF</u>
<u>SCNANFQRIT</u>	<u>DFDAKSGCEP</u>	<u>GGVAYSCADO</u>	<u>TPWAVNDDFA</u>	LGFAATSIAG
SNEAGWCCAC	YELTFTSGPV	AGKKMVVQST	STGGDLGSNH	FDLNIPGGGV
GIFDGCTPQF	GGLPGQRYGG	ISSRNECDRF	PDALKPGCYW	RFDWFKNADN
PSFSFRQVQC	PAELVARTGC	RRNDDGNFPA	VQIPSSSTSS	PVNQPTSTST
TSTSTTSSPP	VQPTTPSGCT	AERWAQ		

FIG._13B

1 mrsslvlfv sawtalaspi rrevsqdlfn qfnlfaqysa aaycgknnda
 51 pagtnitctg nacpevekad atflysfeds gvqdvtfqla ldntnklivl
 101 sfrgsrsien wignlnfdlk eindicsgcr ghdgftsswr svadtlrqkv
 151 edavrehpdy rvvftghslg galatvagad lrgngydidv fsygaprvgn
 201 rafaefltvq tggtylryth tndivprlpp refgyshssp eywiksgtlv
 251 pvtrndivki egidatggnn qpnpdipah lwyfgligtc l

FIG._14B

1 mftpvrrrrvr taalalsaaa alvlgstaas gasatpspap apapapvkqg
 51 ptsvayvevn nnsmlnvgy tladgggnaf dvavifaani nydtgktay
 101 lhfnenvqrv ldnavtqirp lqqqgikvll svlqnhqgag fanfpsqqaa
 151 safakqlsda vakygldgvd fddeyaeygn ngtaqpndss fvhltalra
 201 nmpdkiiisly nigpaasrls yggvdvsdkf dyawnpyygt wqvpqialpk
 251 aqlspaavei grtsrstvad larrtvdegy gvyltynldg gdrtadvsaf
 301 trelygseav rtp

FIG._15B

20 / 28

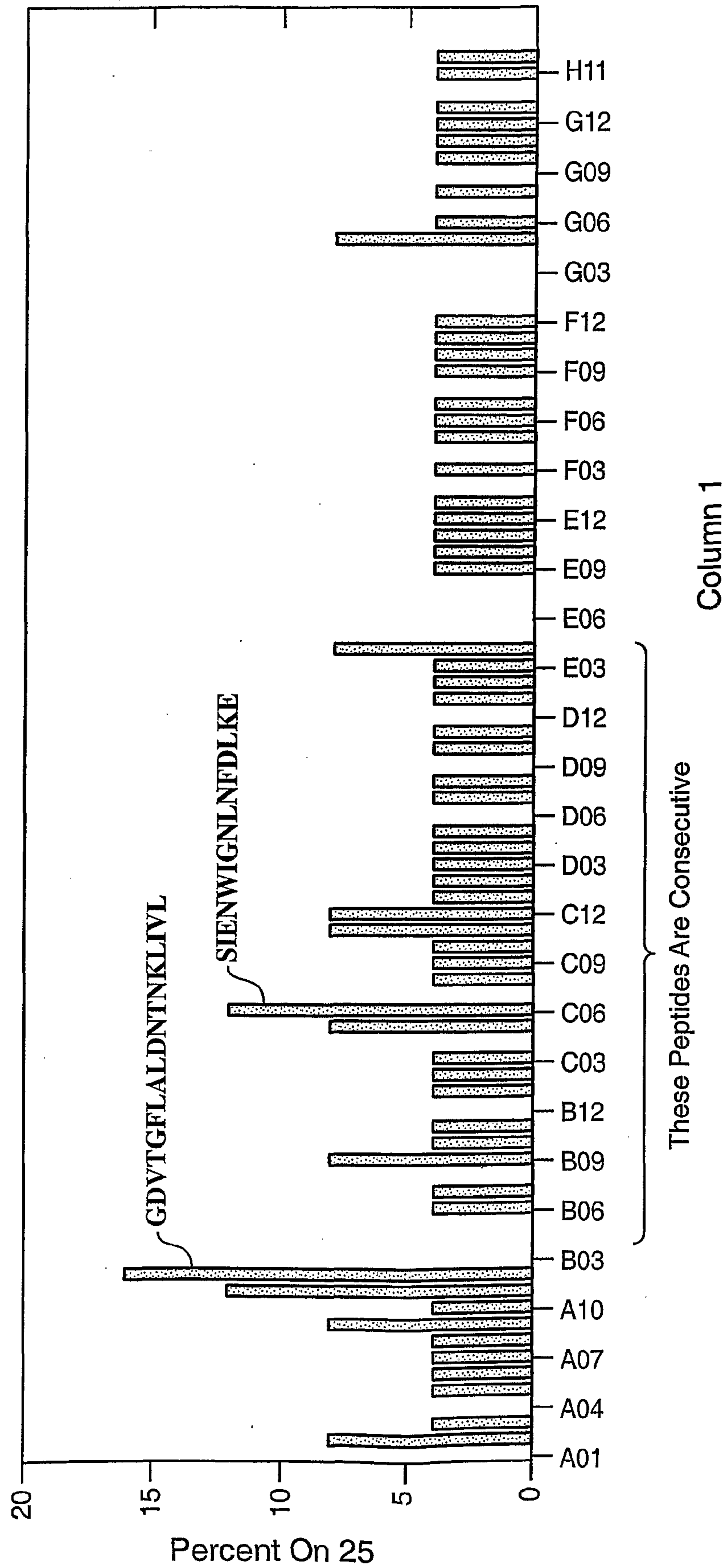


FIG. 14A

21 / 28

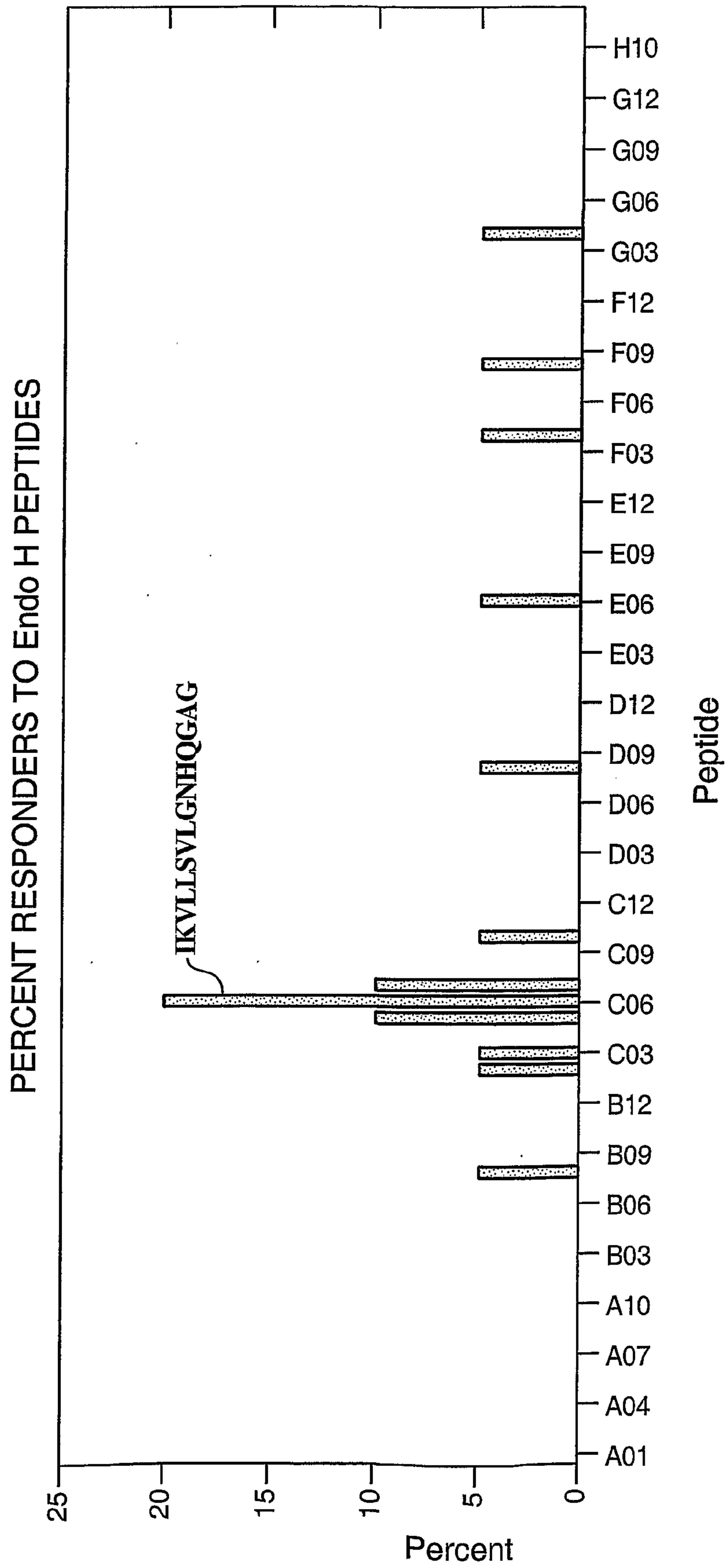


FIG. 15A

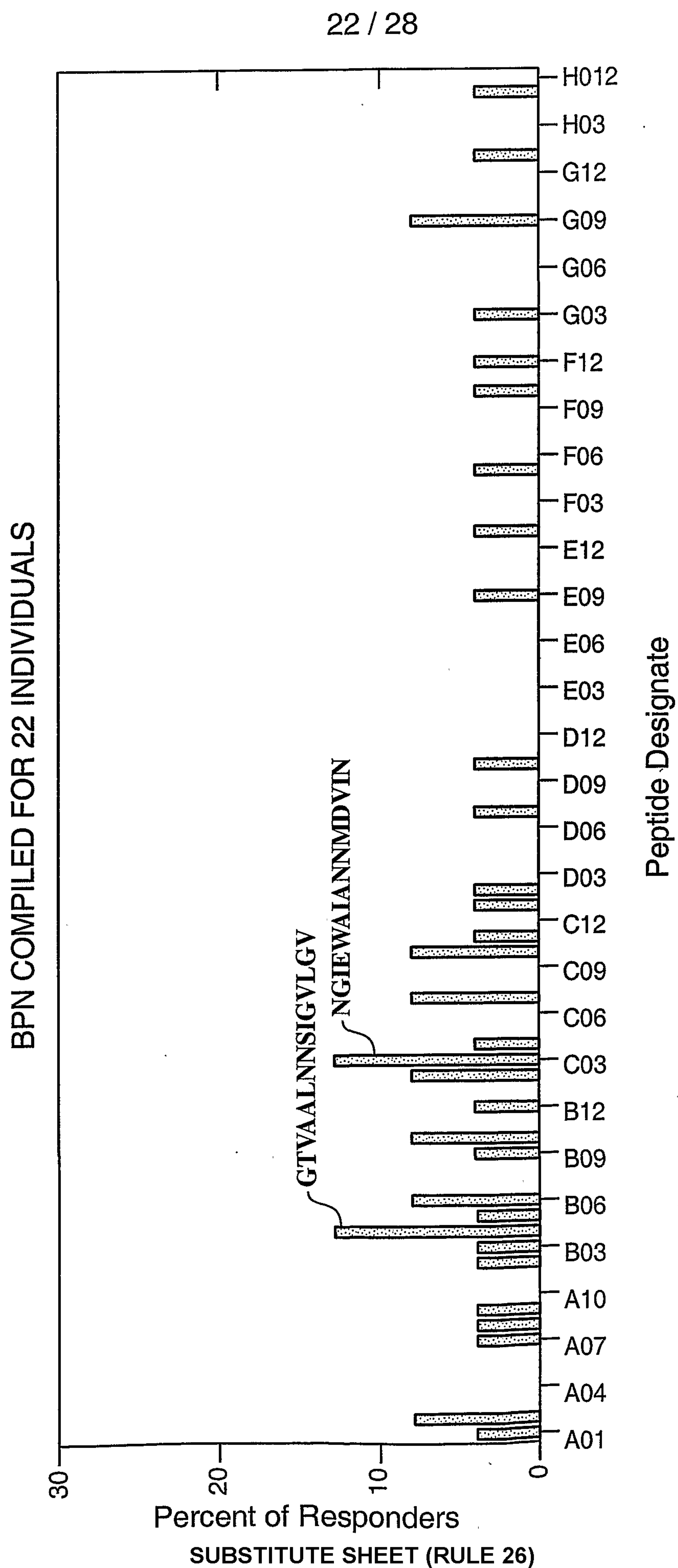


FIG. 16

23 / 28

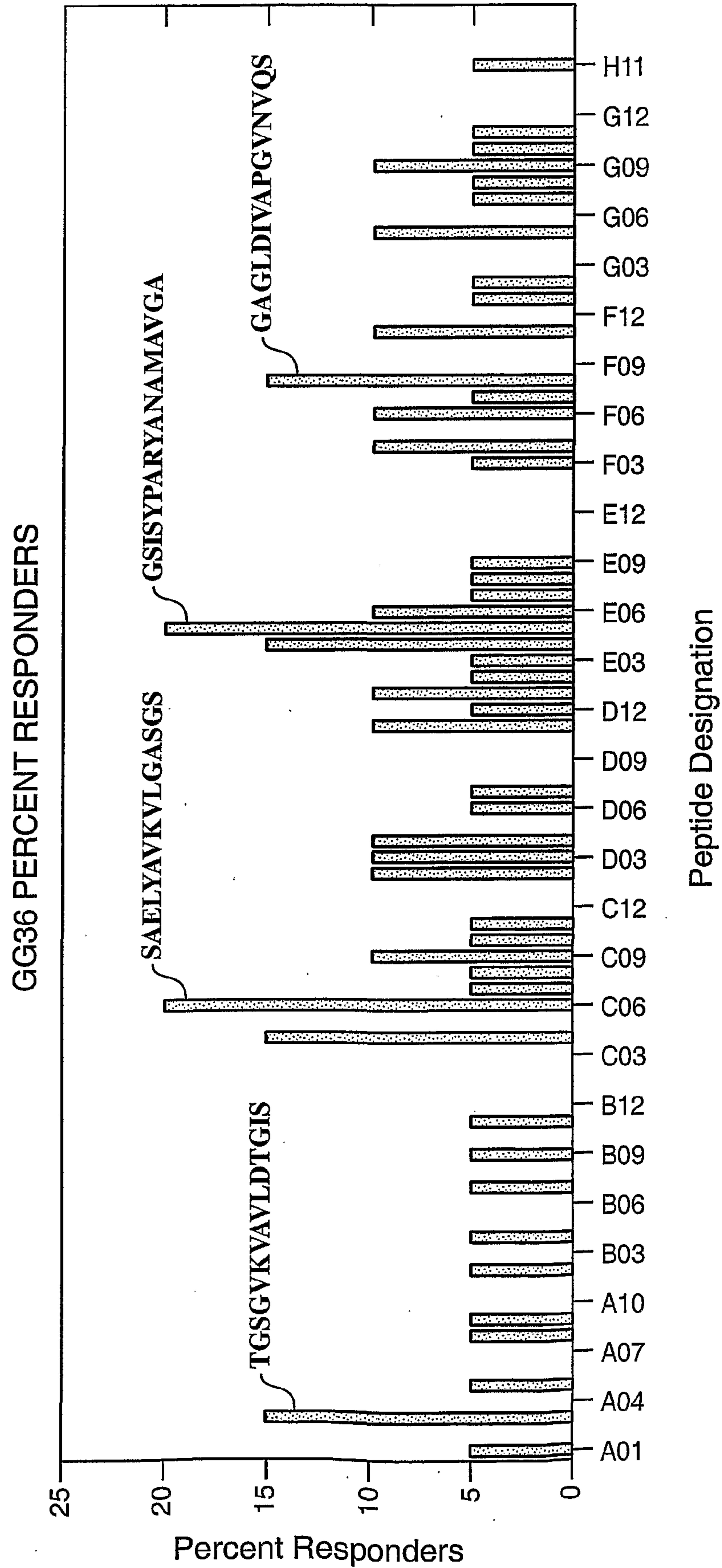


FIG. 17

Hybrid enzyme sequence (GG36-BPN)

GG36

AQSVPWGISRVQAPAAHNRGLTGSQVAVLDTGISTHPDLNIRGGASFVPGEPTQDGNHG

BPN

GTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMHVINMSLGGS

Δ

GSAALKAAVDKAVASGVVVVAAAGNEGTSSTVGYPGKYPSVIAVGAVDSSNQRAFSSVGP

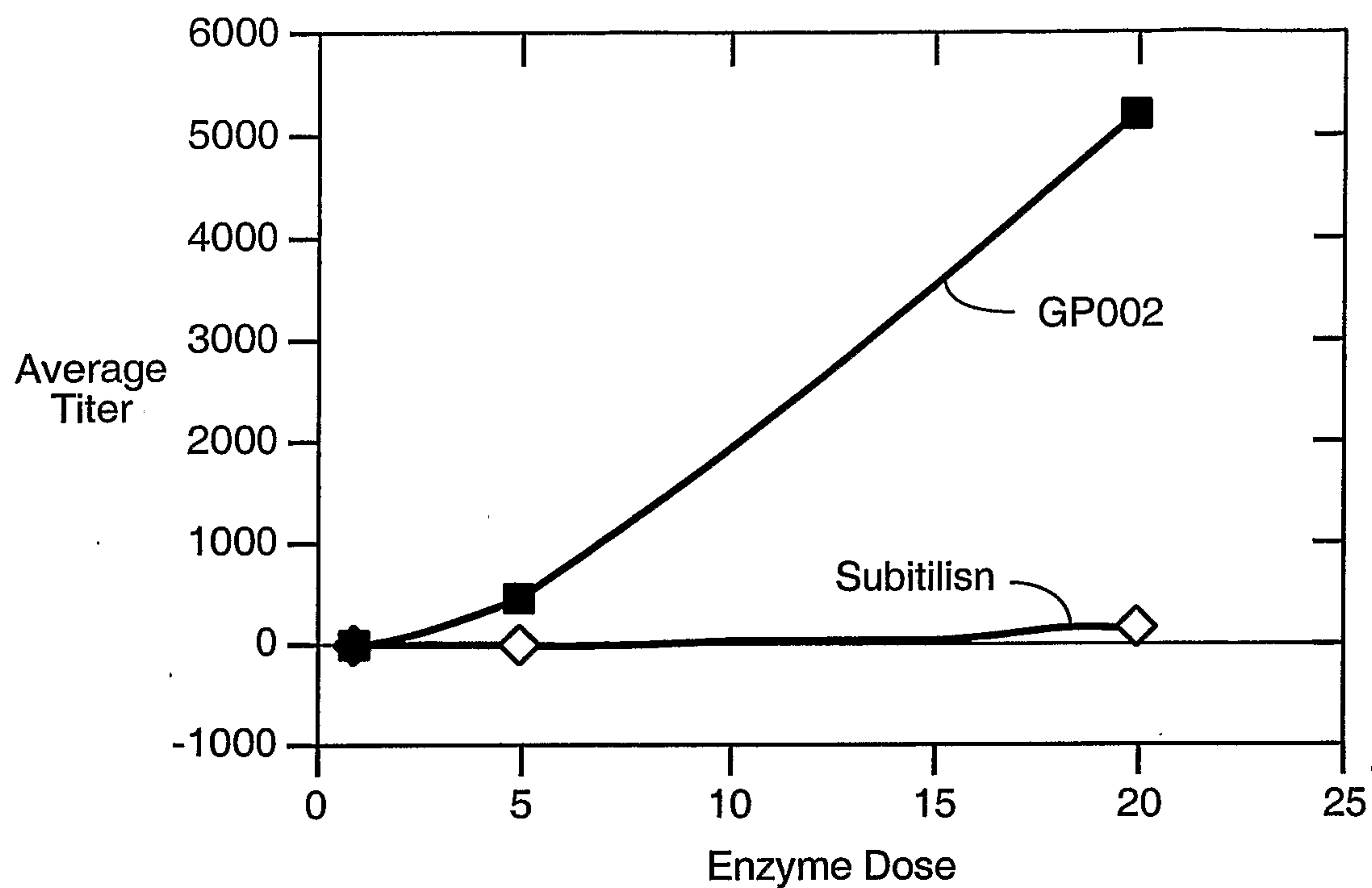
ELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTTKLGD

SFYY GKGLINVQAAQ

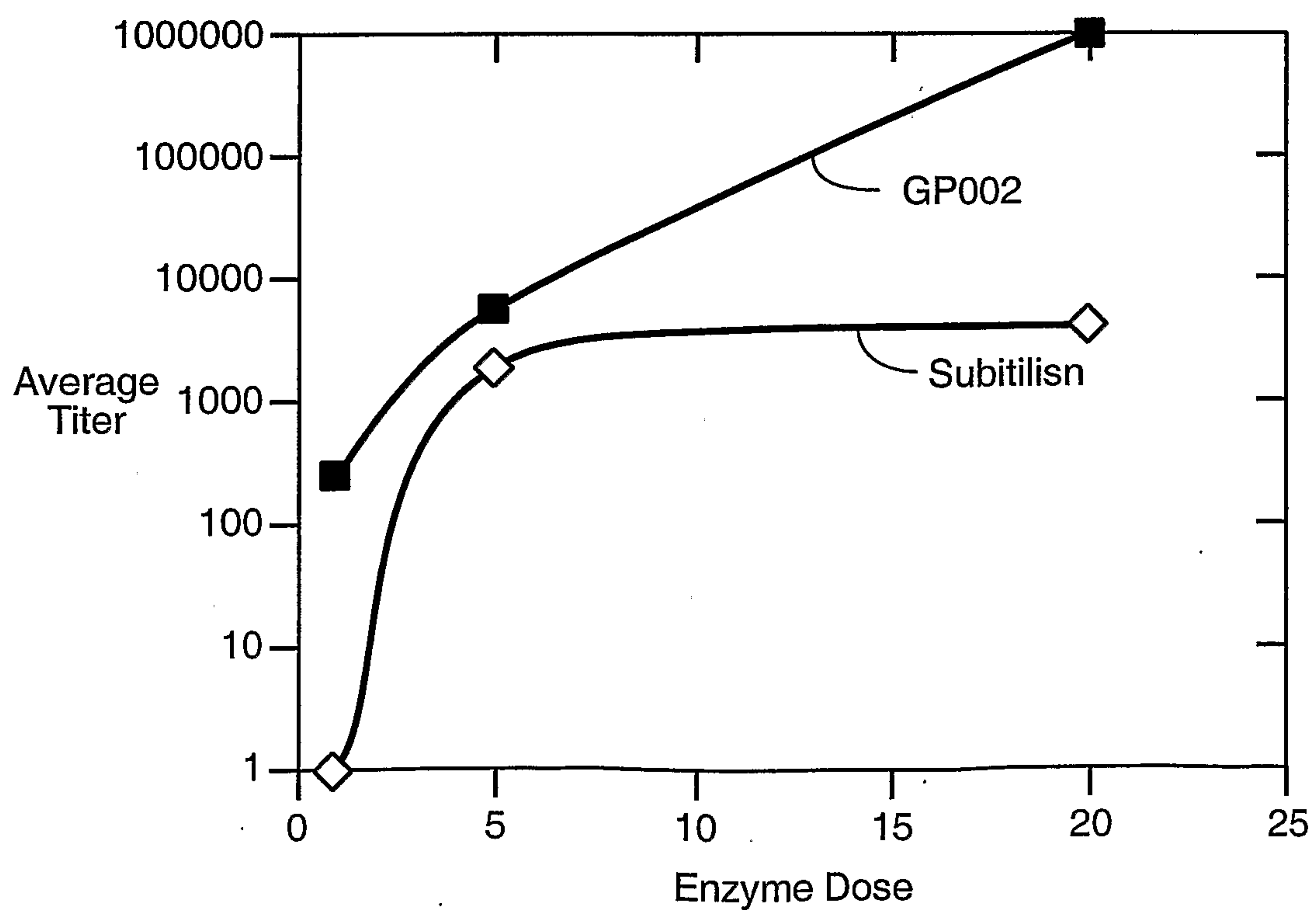
FIG. 18

25 / 28

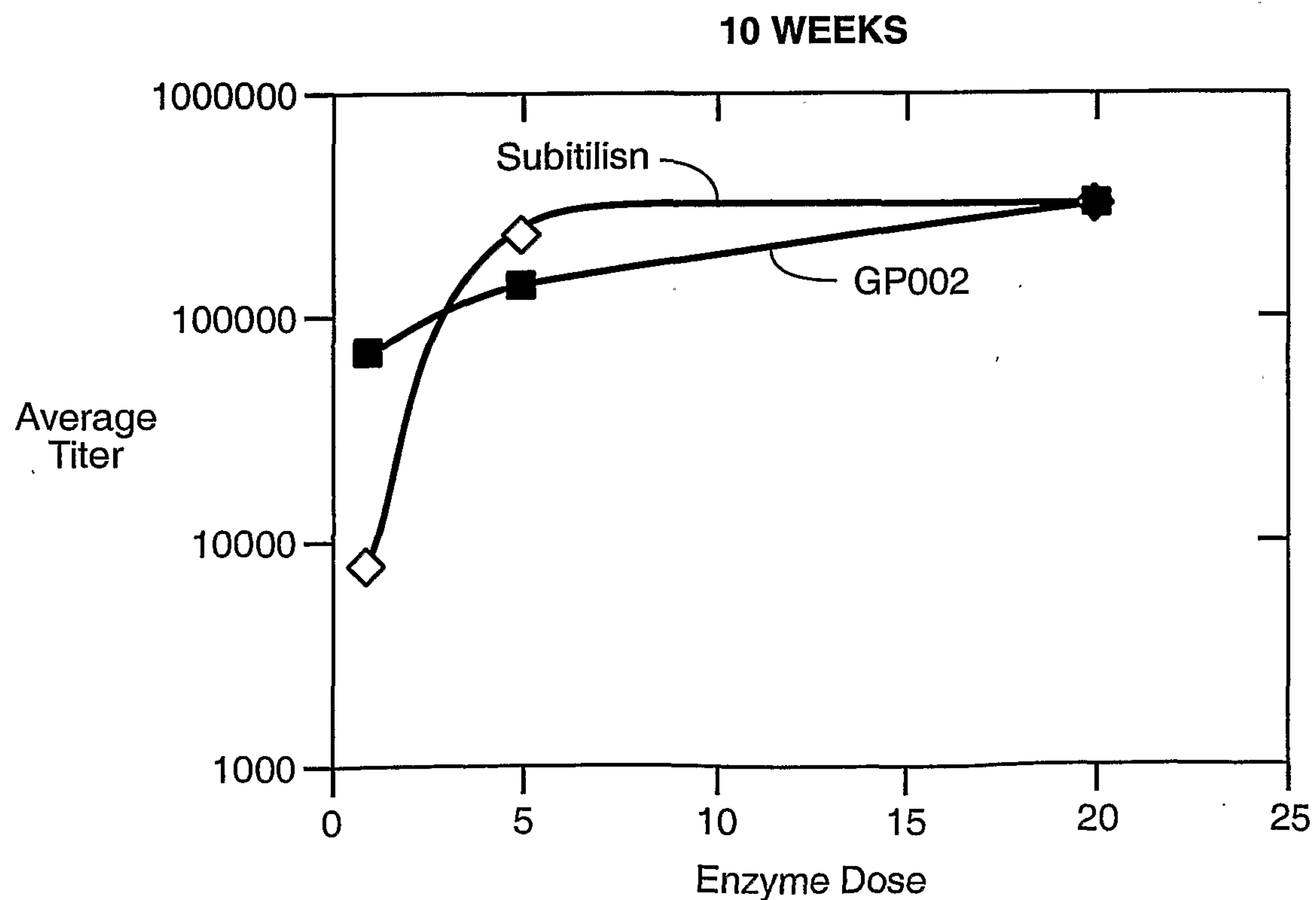
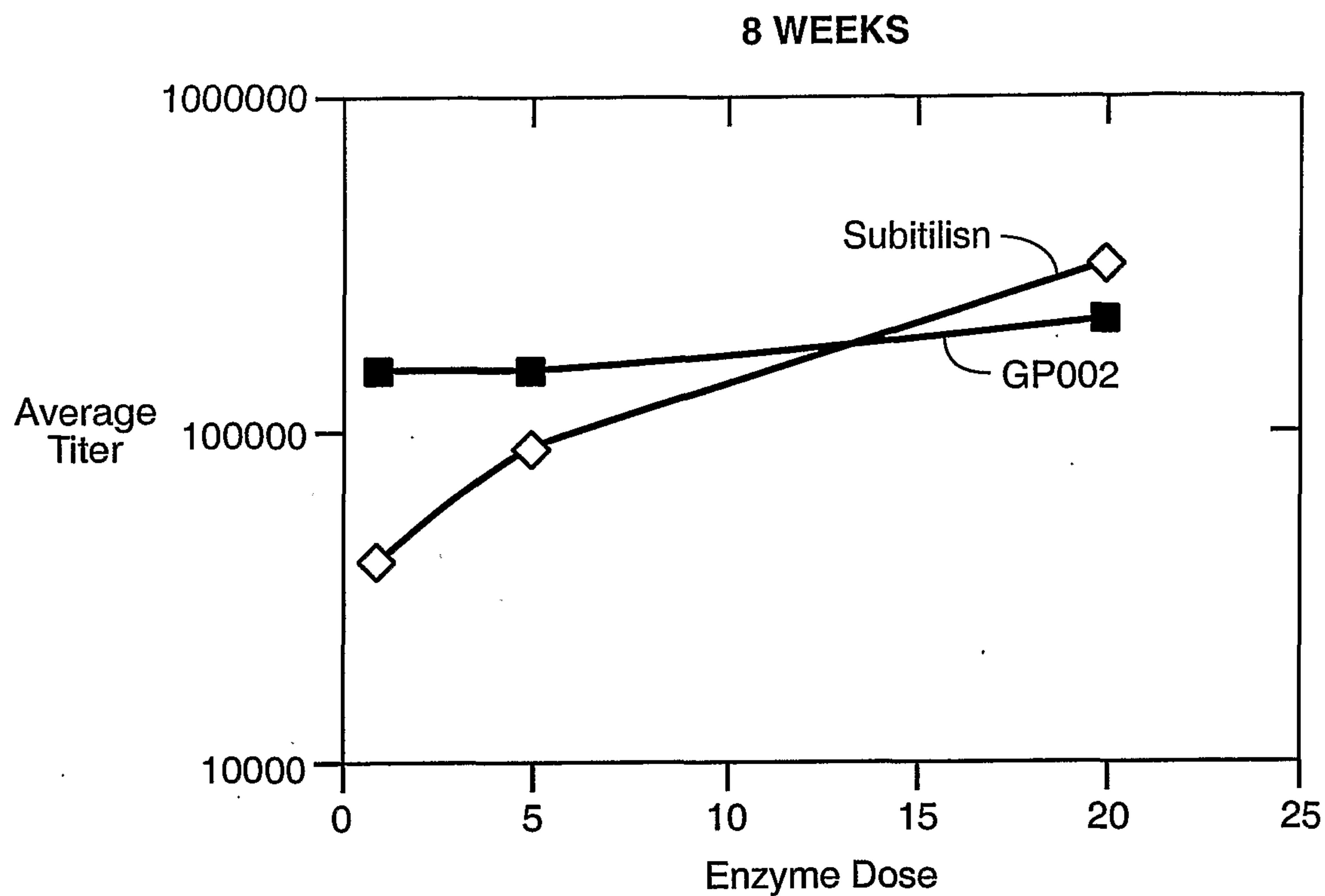
4 WEEKS

**FIG. 19A**

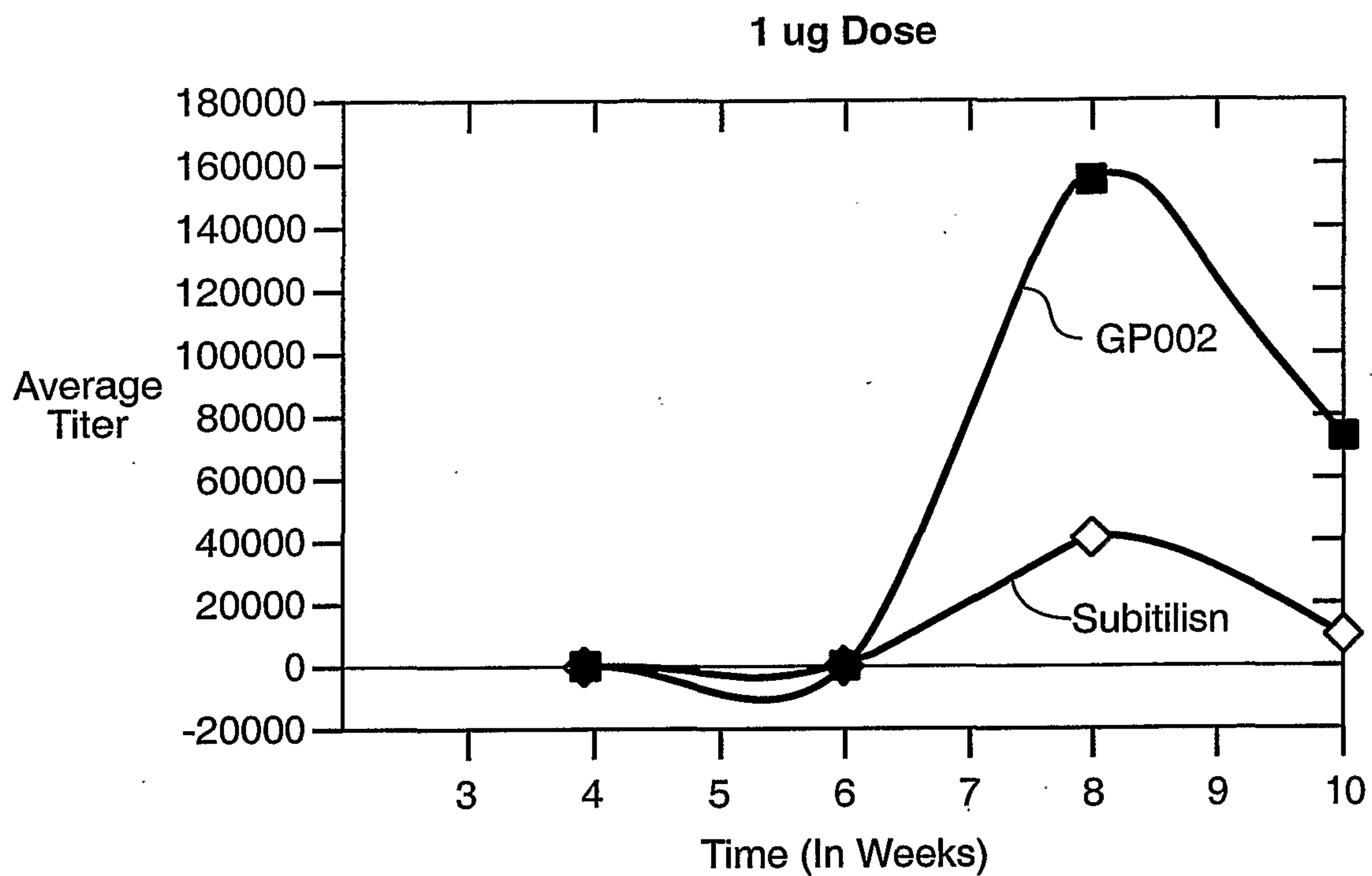
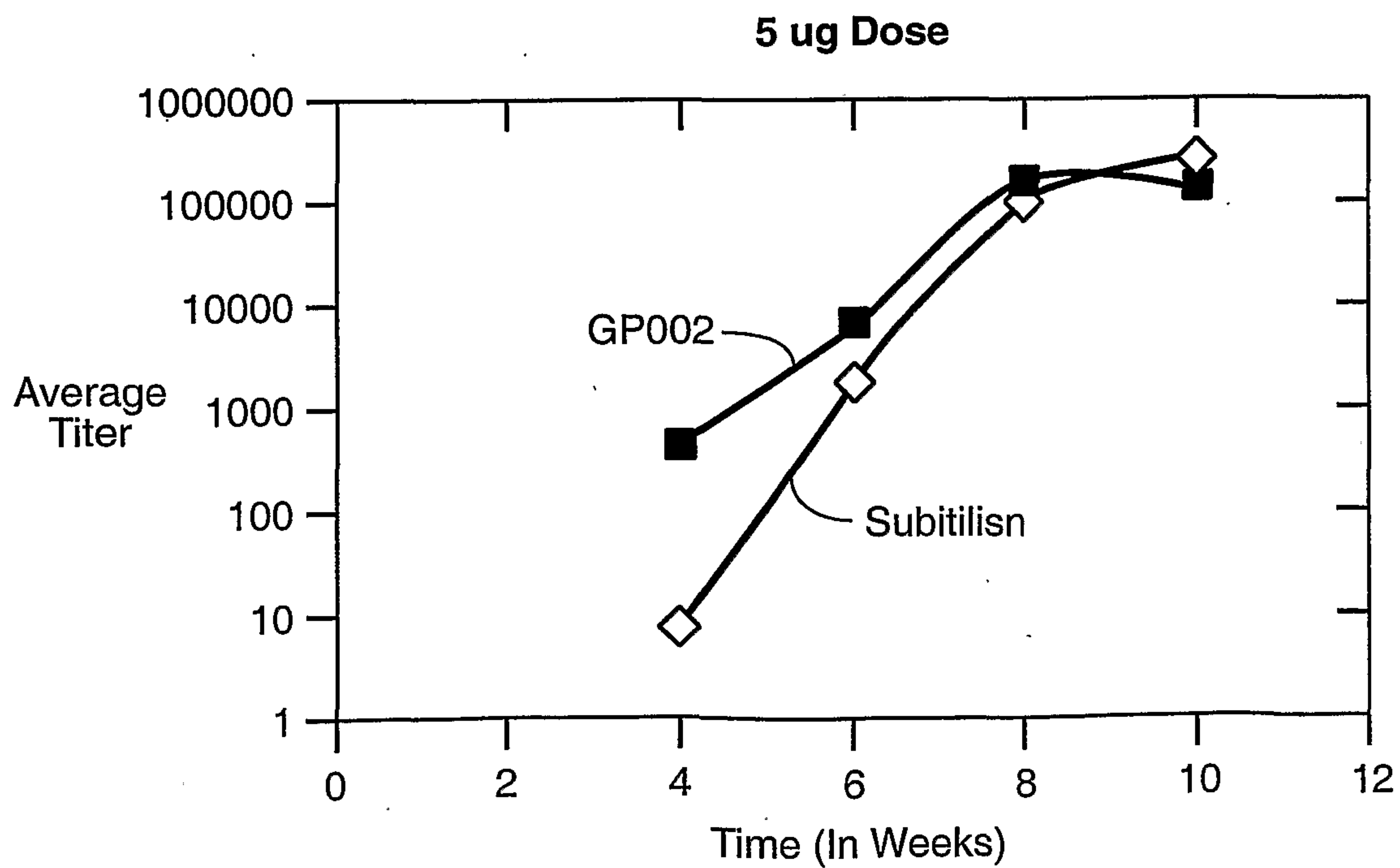
6 WEEKS

**FIG. 19B**

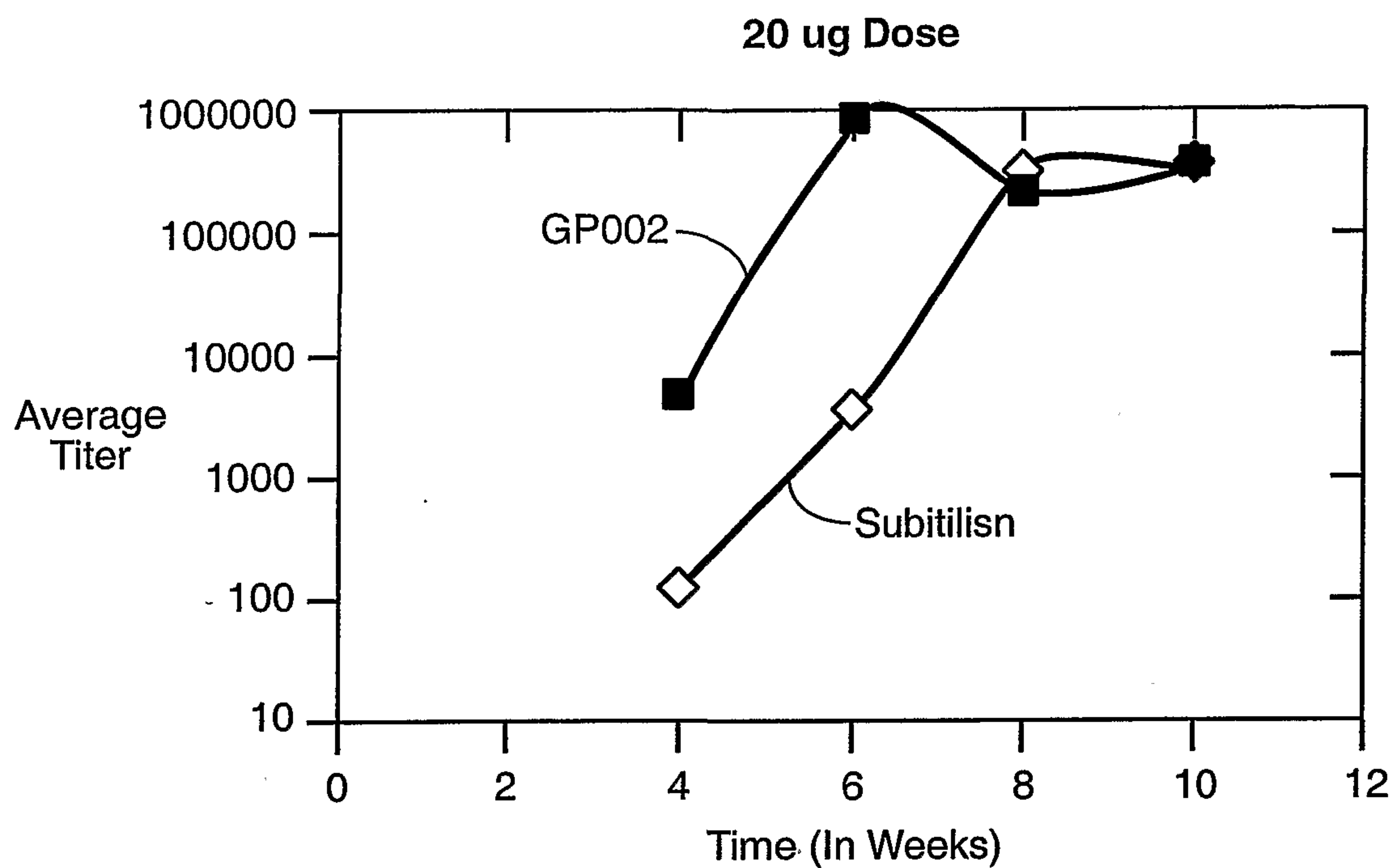
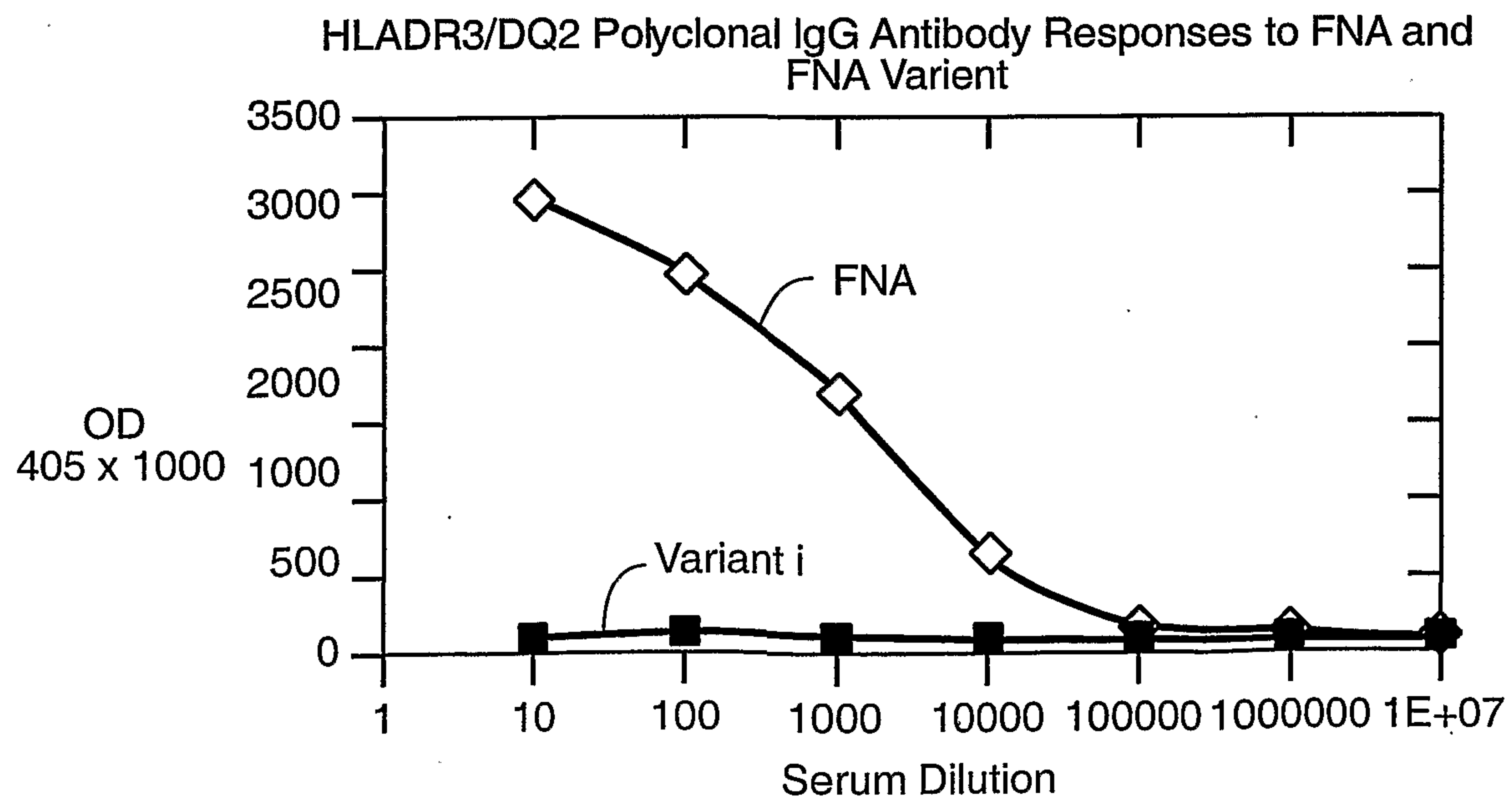
26 / 28



27 / 28

**FIG. 20A****FIG. 20B**

28 / 28

**FIG. 20C****FIG. 21**

HLADR3/DQ2 Polyclonal IgG Antibody Responses to FNA and FNA Variant

