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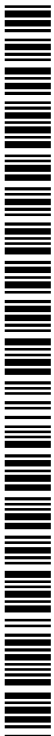


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(54) Title: METHODS, KITS, AND COMPOSITIONS FOR THE DEVELOPMENT AND USE OF MONOCLONAL ANTIBODIES SPECIFIC TO ANTIGENS TRADITIONALLY OF LOW IMMUNOGENICITY

(57) Abstract: The invention relates to the development and use of monoclonal antibodies specific to antigens traditionally of low immunogenicity. The present invention provides methods of producing monoclonal antibodies by chemically conjugating the antigen of interest to a carrier molecule that enables the immune system to respond to immunization with the conjugated antigen. The invention also provides particular compositions of conjugated antigens as well as of monoclonal antibodies specific for those conjugated antigens. The present invention also provides kits for use in detecting disease conditions using the monoclonal antibodies.

**METHODS, KITS, AND COMPOSITIONS
FOR THE DEVELOPMENT
AND USE OF MONOCLONAL ANTIBODIES SPECIFIC
TO ANTIGENS TRADITIONALLY OF LOW IMMUNOGENICITY**

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Field of the invention

The methods and compositions of the invention are in the field of medical biochemistry and relates generally to the development and use of monoclonal antibodies specific to antigens traditionally of low immunogenicity.

Background of the invention

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The immune system has two arms, the adaptive and innate immune responses. These two arms of the immune system work together to combat a foreign invader. Any substance capable of eliciting an adaptive immune response is referred to as an antigen. Foreign molecules can act as antigens and stimulate an immune response resulting in the production of antibodies. Some molecules, however, do not stimulate an immune response. In the past, this was overcome by using an adjuvant, like Freund's complete adjuvant, in order to activate the innate immune system.

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Despite the use of adjuvants, there still remain many molecules, however, that do not elicit an immune response resulting in the production of antigen-specific antibodies. Particularly, many antigens from natural sources often do not elicit a sufficient and molecule-specific immune response. In many instances, chemical synthesis and recombinant technology is used to produce the antigen. These synthetically obtained antigens, however, often do not have the same tertiary structure as the native molecule. When antibodies are developed to the synthetic antigens, the antibodies do not recognize the native molecule.

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The present invention provides novel methods and compositions that allow the production of antigen-specific antibodies to antigens that have traditionally been unable to elicit an adequate and specific immune response. These antibodies are useful for many different applications such as, but not limited to, therapies for disease (such as, but not limited to, cancer, viral infections, etc.), immunodiagnostics, immunochemistry, immunohistology, immunocytology, immunoaffinity chromatography, and genomic and proteomic research.

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Summary of the invention

The present invention relates to methods of producing monoclonal antibodies specific to an antigen of low immunogenicity comprising conjugating the antigen chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the animal, creating a hybridoma from the harvested B cells and screening the hybridomas for

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specificity to the native antigen. In one embodiment, the carrier molecule is HSP70. In another embodiment, the animal has an intact immune system. In yet another embodiment, the animal is a mammal. In further embodiments, B cells are harvested from ascites, spleen, lymph nodes, or blood, either individually or in combination. In yet further embodiments, the hybridoma is created using an immortal cell, such as, but not limited to a mouse myeloma cell, an immortal human cell, or an immortal rat cell. In other embodiments, the screening for specificity is done by a method chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.

The present invention also relates to compositions comprising a monoclonal antibody specific to an antigen of low immunogenicity produced by conjugating the antigen chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity for the native antigen. In a particular embodiment, the carrier molecule is HSP70. In another embodiment, the animal has an intact immune system. In yet another embodiment, the animal is a mammal. In further embodiments, B cells are harvested from ascites, spleen, lymph nodes, or blood, either individually or in combination. In yet further embodiments, the hybridoma is created using an immortal cell, such as, but not limited to a mouse myeloma cell, an immortal human cell, or an immortal rat cell. In other embodiments, the screening for specificity is done by a method chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.

The present invention further relates to methods of producing monoclonal antibodies specific to E7 oncoprotein comprising conjugating the E7 oncoprotein chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity to the native E7 oncoprotein. In another embodiment, the chemical conjugation comprises creating a plasmid with an oligonucleotide sequence encoding E7 oncoprotein and

an oligonucleotide sequence encoding HSP70; and transfecting a host cell with the plasmid, wherein the host cell transcribes the oligonucleotide sequences into the conjugated E7 oncoprotein. In yet another embodiment, the oligonucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 1. In a further embodiment, the oligonucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 3. In yet a further embodiment, the oligonucleotide sequence encoding HSP70 is SEQ ID NO: 5. In other embodiments, the host cell is *E. coli*. In an embodiment, the carrier molecule is HSP70. In another embodiment, the animal has an intact immune system. In yet another embodiment, the animal is a mammal. In further embodiments, B cells are harvested from ascites, spleen, lymph nodes, or blood, either individually or in combination. In yet further embodiments, the hybridoma is created using an immortal cell, such as, but not limited to a mouse myeloma cell, an immortal human cell, or an immortal rat cell. In a particular embodiment, the mouse myeloma cell is a Sp2/0-Ag14 myeloma cell. In other embodiments, the screening for specificity is done by a method chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.

The present invention further provides compositions comprising monoclonal antibodies specific to E7 oncoprotein produced by the method comprising conjugating the E7 oncoprotein chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity to the native E7 oncoprotein. In another embodiment, the chemical conjugation comprises creating a plasmid with an oligonucleotide sequence encoding E7 oncoprotein and an oligonucleotide sequence encoding HSP70; and transfecting a host cell with the plasmid, wherein the host cell transcribes the oligonucleotide sequences into the conjugated E7 oncoprotein. In yet another embodiment, the oligonucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 1. In a further embodiment, the oligonucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 3. In yet a further embodiment, the oligonucleotide sequence encoding HSP70 is SEQ ID NO: 5. In other embodiments, the host cell is *E. coli*. In an embodiment, the carrier molecule is HSP70. In another embodiment, the animal has an intact immune system. In yet another embodiment, the animal is a mammal. In further embodiments, B cells are harvested from ascites, spleen, lymph nodes, or blood, either individually or in combination. In yet further embodiments, the

hybridoma is created using an immortal cell, such as, but not limited to a mouse myeloma cell, an immortal human cell, or an immortal rat cell. In a particular embodiment, the mouse myeloma cell is an Sp2/0-Ag14 myeloma cell. In other embodiments, the screening for specificity is done by a method chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.

10 The present invention also provides for methods of using monoclonal antibodies specific to E7 oncoprotein for the detection of cervical intraepithelial neoplasia comprising obtaining a specimen of cervical epithelial cells and screening the specimen for the presence of E7 oncoprotein. In particular embodiments, the screening method for the presence of E7 oncoprotein is chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay. In another embodiment, the presence of E7 oncoprotein is equal to or greater than 0.05ng/ml. In yet another embodiment, the monoclonal antibodies comprise of at least two immunoglobulin isotypes. In further embodiments, one immunoglobulin isotype is IgG2a, and another is IgG2b. In yet another embodiment, one immunoglobulin isotype has specificity for a different antigenic determinant than the second immunoglobulin isotype.

25 The present invention further provides kits for determining if a subject is at risk for developing cervical intraepithelial neoplasia comprising at least one reagent that specifically detects E7 oncoprotein, and instructions for determining that the subject is at increased risk of developing cervical intraepithelial neoplasia. In an embodiment, the reagent is monoclonal antibodies specific to E7 oncoprotein.

30 The present invention provides as well methods of producing monoclonal antibodies specific to a Prion protein comprising conjugating the Prion protein chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity to the native Prion protein. In particular embodiments, the conjugating is performed chemically using glutaraldehyde. In other embodiments, the carrier molecule is

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HSP70. In another embodiment, the animal is a mouse. In a further embodiment, the screening is done using an enzyme-linked immunosorbent assay.

The present invention further provides kits for determining if a subject is at risk for developing spongiform encephalopathy comprising at least one reagent that specifically
5 detects Prion protein and instructions for determining that subject is at increased risk of developing spongiform encephalopathy.

The present invention also includes methods of producing monoclonal antibodies specific to hyaluronic acid comprising conjugating hyaluronic acid chemically to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from the
10 animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity to the native hyaluronic acid.

The present invention further includes methods of producing monoclonal antibodies specific to matrix metalloprotease 3 comprising conjugating matrix metalloprotease 3 to a carrier molecule, immunizing an animal with the conjugated antigen, harvesting B cells from
15 the animal, creating a hybridoma from the harvested B cells, and screening the hybridomas for specificity to the native matrix metalloprotease 3. In a particular embodiment, the conjugating is performed chemically using glutaraldehyde. In another embodiment, the carrier molecule is HSP70. In yet another embodiment, the animal is a mouse. In yet another embodiment, the screening is done using an enzyme-linked immunosorbent assay.

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Description of figures

Figure 1 shows how HSP70 facilitates the presentation of antigens.

Figure 2 shows a flow chart showing the pathology of Human Papilloma Virus. CIN refers to cervical intraepithelial neoplasia. HSV refers to herpes simplex virus. HLA refers to human leukocyte antigen.

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Figure 3 shows the sequences of the primers for E7 gene amplification using PCR (SEQ ID NOS: 14-17).

Figure 4 shows the oligonucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences for clone E7 oncoprotein from HPV16 with EcoRI and BamHI restriction sites.

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Figure 5 shows the oligonucleotide (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences for clone E7 oncoprotein from HPV18 with EcoRI and BamHI restriction sites.

Figure 6 shows a construct of the position where the HPV16 E7 and alternatively where the HPV18 E7 gene was inserted at the EcoRI-BamHI site in a pBluescript SK (+) (Stratagene) plasmid (SEQ ID NO: 18).

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Figure 7 shows a picture of an electrophoresis gel demonstrating isolated bands of E7 oncoprotein from *E. coli* lysates transfected with HPV16 E7 and HPV18 E7 genes.

Figure 8 shows the DNA templates (SEQ ID NOs.: 19-22) used for PCR amplification of DNA containing E7 genes. The templates are based on plasmid DNAs pHE716 and pHE718, which contain the HPV E7 genes.

Figure 9 shows a schematic diagram of the structure of the plasmid PQE30-E716-dnaK.

Figure 10 shows the oligonucleotide sequence for Plasmid pQE30-dnaK (SEQ ID NO: 5).

Figure 11 shows a calibration curve for the quantitative determination of HPV16 E7 oncoprotein under optimum conditions.

Detailed description of the invention

Definitions:

To facilitate understanding of the invention, a number of terms are defined below.

As used herein, the term “chemically conjugated,” or “conjugating chemically” refers to linking the antigen to the carrier molecule. This linking can occur on the genetic level using recombinant technology, wherein a hybrid protein may be produced containing the amino acid sequences, or portions thereof, of both the antigen and the carrier molecule. This hybrid protein is produced by an oligonucleotide sequence encoding both the antigen and the carrier molecule, or portions thereof. This linking also includes covalent bonds created between the antigen and the carrier protein using other chemical reactions, such as, but not limited to glutaraldehyde reactions. Covalent bonds may also be created using a third molecule bridging the antigen to the carrier molecule. These cross-linkers are able to react with groups, such as but not limited to, primary amines, sulfhydryls, carbonyls, carbohydrates or carboxylic acids, on the antigen and the carrier molecule. Chemical conjugation also includes non-covalent linkage between the antigen and the carrier molecule.

As used herein, the term “carrier molecule” refers to any molecule that is chemically conjugated to the antigen of interest that enables an immune response resulting in antibodies specific to the native antigen.

As used herein, the terms “native,” “natural” “native antigen,” or “natural antigen” refers to the antigen as it occurs in nature. With respect to the invention, the “native antigens” are of “low immunogenicity.” “Low immunogenicity” refers to the inability of the natural molecule to elicit a strong immune response resulting in the production of high affinity antibodies. The term “antigen”, “antigen of interest,” or specific molecules, such as—but not limited to E7 oncoprotein, Prion protein, matrix metalloprotease 3, hyaluronic acid—include the whole molecule or any portions thereof that maintain antigenic distinctiveness specific for the native antigen.

As used herein, the term “intact immune system” refers to an animal with a functional immune system capable of raising an immune response to a foreign antigen. The immune response includes the ability to generate B cells that secrete antibodies.

As used herein “amino acid sequence” refers to an amino acid sequence of a naturally occurring protein molecule, “amino acid sequence” and like terms, such as “polypeptide” or “protein” are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

As used herein, the terms “nucleic acid molecule encoding,” “DNA sequence encoding,” and “DNA encoding” refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

DNA molecules are said to have “5’ ends” and “3’ ends” because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5’ phosphate of one mononucleotide pentose ring is attached to the 3’ oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotides or polynucleotide, referred to as the “5’ end” if its 5’ phosphate is not linked to the 3’ oxygen of a mononucleotide pentose ring and as the “3’ end” if its 3’ oxygen is not linked to a 5’ phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5’ and 3’ ends. In either a linear or circular DNA molecule, discrete elements are referred to as being “upstream” or 5’ of the “downstream” or 3’ elements. This terminology reflects the fact that transcription proceeds in a 5’ to 3’ fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5’ or upstream of the coding region. However, enhancer elements can exert their effect even when located 3’ of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3’ or downstream of the coding region.

As used herein, the terms “an oligonucleotide having a nucleotide sequence encoding” and “polynucleotide having a nucleotide sequence encoding,” means a nucleic acid sequence comprising the coding region of a gene or, in other words, the nucleic acid sequence that encodes a gene product. The coding region may be present in a cDNA, genomic DNA, or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper

initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, etc. or a combination of both endogenous and exogenous control elements.

As used herein, the term “regulatory element” refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements include splicing signals, polyadenylation signals, termination signals, etc.

“Amplification” is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (*i.e.*, replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (*i.e.*, synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of “target” specificity. Target sequences are “targets” in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Q replicase, MDV-1 RNA is the specific template for the replicase [D.L. Kacian *et al.*, *Proc. Natl. Acad. Sci. USA*, 69:3038 (1972)]. Other nucleic acids will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters [Chamberlin *et al.*, *Nature*, 228:227 (1970)]. In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction [D.Y. Wu and R. B. Wallace, *Genomics*, 4:560 (1989)]. Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences [H.A. Erlich (ed.), *PCR Technology*, Stockton Press (1989)].

As used herein, the term “amplifiable nucleic acid” is used in reference to nucleic

acids that may be amplified by any amplification method. It is contemplated that “amplifiable nucleic acid” will usually comprise “sample template.”

As used herein, the term “sample template” refers to nucleic acid originating from a sample that is analyzed for the presence of “target” (defined below). In contrast, “background
5 template” is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is most often inadvertent. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

10 As used herein, the term “primer” refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*,
15 in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact
20 lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

As used herein, the term “probe” refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to
25 another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labelled with any “reporter molecule,” so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent,
30 radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the term “target,” when used in reference to the polymerase chain reaction, refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the “target” is sought to be sorted out from other nucleic acid
35 sequences. A “segment” is defined as a region of nucleic acid within the target sequence.

As used herein, the term “polymerase chain reaction” (“PCR”) refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for
5 amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary
10 sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one “cycle”; there can be numerous “cycles”) to obtain a high concentration of an amplified segment of the desired target sequence. The length of the
15 amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the “polymerase chain reaction” (hereinafter “PCR”). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they
20 are said to be “PCR amplified.”

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ³²P-labeled deoxynucleotide triphosphates, such as
25 dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

As used herein, the terms “PCR product,” “PCR fragment,” and “amplification
30 product” refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

As used herein, the term “amplification reagents” refers to those reagents
35 (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along

with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

As used herein, the terms “restriction endonucleases” and “restriction enzymes” refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide
5 sequence.

As used herein, the term “recombinant DNA molecule” as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

The term “western blot” refers to the analysis of protein(s) (or polypeptides)
10 immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabelled antibodies.

The term “antigenic determinant” as used herein refers to that portion of an antigen
15 that makes contact with a particular antibody (*i.e.*, an epitope). When a protein or fragment of a protein, or chemical moiety is used to immunize a host animal, numerous regions of the antigen may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as
20 antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the “immunogen” used to elicit the immune response) for binding to an antibody.

As used herein, the term “vector” is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The term “vehicle” is sometimes used interchangeably with “vector.”

The term “expression vector” as used herein refers to a recombinant DNA molecule
25 containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences.
30 Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals.

As used herein, the term host cell refers to any eukaryotic or prokaryotic cell (*e.g.*, bacterial cells such as *E. coli*, yeast cells, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*. For example, host cells
35 may be located in a transgenic animal.

The term "transfection" as used herein refers to the introduction of foreign DNA into eukaryotic cells. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics.

I. Certain Antigens Have Low Immunogenicity

There are many low-molecular-weight compounds, which are not capable of independently eliciting an immune response because of their small size. In an embodiment, a hapten can be conjugated with a carrier protein (such as, but not limited to, bovine serum albumin, egg albumin, or keyhole limpet hemocyanin), and this conjugate is used for immunization. In such embodiments, a variety of problems arise. For example, the primary immune response may be directed at the carrier protein and not at the hapten molecule, and this must be considered when testing sera from immunized animals and hybridoma supernatants. The testing should reveal whether the antibodies are specific to the hapten or to the conjugate as a whole.

Another problem can include changes in the three-dimensional structure (conformation) of the hapten that can occur during conjugation with the carrier. This may result in the antibodies being specific to the hapten within the conjugate, but are incapable of recognizing the free hapten molecule. In such cases, different conjugation variants can be tried such that different functional groups of the hapten are exposed on the carrier surface.

In yet another embodiment, immunization with immune complexes can be used when the antigen of interest is either a conservative low-immunogenic protein or is a minor antigenic determinant that already has a very low percentage of all specific antibodies directed to it during the immune response. In the first instance, the antibody component of the immune complex may be monoclonal antibodies with an unsatisfactory affinity obtained earlier after typical immunization. In the second case, monoclonal antibodies against strong antigenic determinants are used. An example of the successful use of this approach is the production of antibodies against human erythropoietin, α and γ interferons, and immunoglobulins of closely related species (such as, but not limited to, rat monoclonal antibodies against mouse IgG). *See* Table 1.

In other embodiments, some antigens have a very high degree of conservation and are incapable of eliciting any noticeable immune response, despite their protein nature and sufficient molecular weight (for example, but not limited to, hemoglobin, certain enzymes). In these particular embodiments, the antigens may be conjugated with the heat shock protein (such as, but not limited to, heat shock protein 70 kDa, otherwise known as HSP70). *See*

Table 1, indicated as APP conjugate. The data shows that HSP70 facilitates the presentation of peptide fragments by exposing them in a certain way (*see Fig. 1*). Conjugation of proteins and peptides with HSP70 usually results in an abrupt intensification in the production of antibodies against these proteins and peptides. Yet, in other particular embodiments, the complete suppression of the immune response was seen with the conjugation of HSP70–interferon γ .

II. Using HSP to Increase Immunogenicity

The methods described herein include a novel use of heat-shock protein as a vehicle to present the antigen of interest to produce antigen-specific antibodies for antigens that normally do not elicit an adequate immune response for the native molecule. Cells have evolved many mechanisms to help them survive in an unpredictable and hazardous world. One such mechanism is the heat-shock response, which is evoked by the exposure of cells to unusually high temperatures (such as, but not limited to, a fever), and other stresses including hypoxia, nutrient deprivation, oxygen radicals, metabolic disruption, viral infection, phagocytosis and transformation. This heat shock response includes the production of heat-shock proteins (“HSPs”). One such family of proteins is the HSP70 proteins. Members of the HSP70 protein family are found to function in the cytosol, mitochondria, as well as the endoplasmic reticulum of cells. HSP70 proteins each work with a small set of associated proteins when they help other proteins to fold. HSP70 and its associated proteins tend to bind to hydrophobic amino acids on a protein’s surface, where they hydrolyze ATP, often binding and releasing their protein with each cycle of ATP hydrolysis. These repeated cycles help the target protein, for example, to refold.

Some of these heat-shock proteins help stabilize and repair partly denatured cell proteins. These HSPs also act as molecular chaperones. As chaperones, HSPs aid the conversion of a molten globule form of a protein to the correctly folded final compact conformation of the protein, protecting functional proteins from degradation, shuttling abnormal proteins to areas of degradation, transferring proteins between different intracellular compartments, assisting in the assembly of protein multimers, or aiding in the presentation of antigens with major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells.

It has been shown that the immune system responds vigorously to the heat-shock proteins of microbes, such as bacteria, parasites and fungi. The presence of foreign HSP triggers a prompt and potent immune response by the body. [Murray, P., Young, R., *J. Bacteriol.* 174:4193-4196 (1992)]. HSP70 is a major target of the immune response to many different pathogens including bacteria, fungi, helminth, and protozoan parasites. [Young, R.

A., and Elliot, T. J., *Cell*, 59:5-8 (1989); Kaufmann, S.H., *Immunol. Today*, 11:129-136, (1990); Young, D.B., *et al.*, *Stress proteins and infectious diseases*, p. 131-165 in *Stress proteins in biology and medicine*, Morimoto, R.I., *et al.*, (eds.) Cold Spring Harbour Laboratory, Cold Spring Harbor, NY (1990); Young, R.A., *Ann. Rev. Immunol.*, 8:401-420(1990)]. Immunization with a variety of pathogen HSPs induces a strong immune response and provides protection against diseases caused by these pathogens. [Suzue, K. and Young, R.A., *J. Immunol.*, 156:873-876 (1996)].

The use of HSPs as vaccine vehicles based on the body's strong immune response began in the early 1990's. [Udono, H. and Srivastava, P.K., *J. Exp. Med.*, 178:1391-1396, (1993); Udono, H., *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:3077-3081, (1994); Suto, R. and Srivastava, P.K., *Science*, 269:1585-1588, (1995); Blachere, N.E., *et al.*, *J. Exp. Med.*, 186:1315-1322, (1997); Tamura, Y. P., *et al.*, *Science*, 278:117-120, (1997); Nair, S., *et al.*, *J. Immunol.*, 162:6426-6432 (1999)]. The chemical conjugation [Lussow, A.R., *et al.*, *Eur. J. Immunol.* 21:2297-2302 (1991); Barrios, C., *et al.*, *Eur. J. Immunol.*, 22:1365-1372, (1992); and Perraut, R., *et al.*, *Clin. Exp. Immunol.*, 93:382-386 (1993); Suzue, K. and Young, R.A., *J. Immunol.*, 156:873-876, (1996); Suzue, K., *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:13146-13151, (1997); Rico, A.I., *et al.*, *Infect. Immun.*, 66:347-352, (1998), hereby incorporated by reference] of antigens to HSP70 can create potent and customized immunogens that can elicit MHC class I-restricted, CD8+ cytotoxic T cell responses sufficient to mediate rejection of tumors expressing the fusion partner. This same cellular machinery is employed in the present invention in order to create antigen specific antibodies specific to native unconjugated antigen. See **Fig. 1**.

The prior art describes the use of HSP70 for the stimulation of an immune response whereby stimulating activity was assessed according to the degree of cellular and humoral immunity induction. There is, however, a significant difference between the induction of antibody genesis and monoclonal antibody production. The presence of antibodies in the serum does not always lead to the production of high affinity monoclonal antibodies that are able to recognize natural molecules. In a particular embodiment, in producing monoclonal antibodies to E7 oncoproteins, antibody genesis was observed after immunization of mice with the purified recombinant protein, however, upon testing none of the clones recognized natural E7 in cell line lysates containing the HPV genome. The immune system appears to recognize some determinants of the recombinant proteins that don't exist in natural proteins. In other words, the antibodies produced reacted with the antigen used for immunization, but did not react with the natural protein.

The present invention is not limited to any particular mechanism, however, it is

believed that the three-dimensional protein structure of the recombinant proteins was different from that of the natural proteins. Therefore, non-natural antigenic determinants would be presented to the immune system. In overcoming this problem, the present invention uses a natural mechanism of antigen presentation (using hybrid proteins including the protein or antigen of interest conjugated with heat shock proteins) to present natural conformational determinants to the immune system. In particular embodiments, this is accomplished by using HSP70.

In these particular embodiments, an antigen of interest is conjugated to HSP70 or any portion of HSP70 that allows the antigen of interest to be immunogenic. Conjugation may be achieved chemically. [Lussow, A.R., *et al.*, *Eur. J. Immunol.* 21:2297-2302 (1991); Barrios, C., *et al.*, *Eur. J. Immunol.*, 22:1365-1372, (1992); and Perraut, R., *et al.*, *Clin. Exp. Immunol.*, 93:382-386 (1993); Suzue, K. and Young, R.A., *J. Immunol.*, 156:873-876, (1996); Suzue, K., *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:13146-13151, (1997); Rico, A.I., *et al.*, *Infect. Immun.*, 66:347-352, (1998)]. An antigen may be any protein, peptide, nucleotide sequence, or chemical moiety. HSP70 can be from any source including but not limited to, mammalian, reptilian, amphibian, ichthyoidal, avian, insectoid, bacterial, fungal, helminthian, protozoan, viral, and plant.

Once the antigen of interest is conjugated to an HSP70 molecule, the conjugate is introduced to a foreign intact immune system able to process the conjugate and raise an immune response to the immunization with the conjugate. Any animal can be immunized and is not limited to mammals, but typical laboratory examples include, rat, mouse, hamster, gerbil, guinea pig, rabbit, dog, monkey, chicken, goat, sheep, swine, camel, cow, horse, and cat.

III. Immunization

The effectiveness of immunization directly determines whether high affinity monoclonal antibodies can be produced. Various techniques are well known in the art and are used to increase the effectiveness of immunization, some of which are described herein.

A. Selecting the Subject for Immunization

In particular embodiments, there are three typical types of hybridoma systems: mouse, rat, and human. The mouse hybridoma is the most widely used, and the human hybridoma the least used. The invention is in no way limited to these examples. Any animal with an intact immune system may be used as a B cell source for hybridization with a myeloma or any other immortal cell, including but not limited immortal human and immortal rat cells, to create a hybridoma.

The BALB/c mouse line is used most often for the immunization of mice; all mouse

myeloma lines originate from this line. In the case of low-immunogenic antigens, mice of another line can be immunized. These mice may produce a stronger immune response to certain antigens in comparison with BALB/c. First generation offspring, obtained by crossing BALB/c and the line used for immunization, can be used to grow ascites for hybridoma production.

In other embodiments, rats are used, the literature emphatically recommends the LOU/C line, but totally satisfactory results were obtained with rats from the WISTAR line.

B. Mouse Immunization Schedules

1. Short immunization schedule

In particular embodiments, the antigen is administered twice with a 2-week interval into the hind footpads Balb/c mice. In yet further embodiments, the amount of the antigen typically ranges from 5 to 200 µg per mouse (but is not limited to these ranges) depending on its availability and immunogenicity. For toxic antigens, the dose may be reduced to as low as 0.1 µg. For the first administration of antigen (round of immunization), the antigen solution ranges typically from 50-200 µl in volume (but is not limited to this particular range so long as an immune response is achieved) and is combined with with an adjuvant such as complete Freund's adjuvant ("CFA"). CFA is commonly known in the art and is as mineral oil with a suspension of killed mycobacteria. Other adjuvants may also be used, such as, but not limited to, as mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially human adjuvants such as BCG ("Bacille Calmette-Guerin") and *Corynebacterium parvum*.) Vigorous mixing of the antigen solution with CFA produces a finely dispersed emulsion for delaying and prolonging the entry of the antigen into tissue. The second round of immunization is carried out as the first, but with the use of incomplete Freund's adjuvant (IFA), which contains no mycobacteria.

In yet further embodiments, with antigens of low immunogenicity, it is useful to immunize a group of animals with different antigen doses and using various immunization schedules. In a particular embodiment, on the third or fourth day after the second round of immunization, blood samples are taken and analyzed for their content of antibodies against the specified antigen. Animals with the highest titer are usually selected for analysis. There are, however, exceptions such as protein toxins used as antigens. These types of antigens may produce a high specific antibody titer, but is also combined with a high percentage of B lymphocytes killed by the toxin. In these cases, animals that do not have the highest titer must be selected and/or attention must be focused on the viability of cells for fusion. On the fourth day after the second round of immunization, the animal is sacrificed and the cells of popliteal

lymph nodes are fused with myeloma cells. Other lymph nodes from the animal may also be used.

2. Long immunization schedules

In another embodiment, where the short immunization schedule is not effective, various types of long schedules can be tried. In this case, the animals are usually immunized at an interval of 2-4 weeks either intraperitoneally or subcutaneously (alternatively, but less often, intravenously or orally). CFA is used for the first round of immunization and IFA for the next one.

On days 10-14 after each round of immunization (beginning with the second round), blood from the immunized animals is tested for the content of specific antibodies as well known in the art. If the antibody titer has reached the desired level, the last round of immunization with the antigen (called boosting) is given intravenously without adjuvants. In preferred embodiments, 1/10 of the original dose is given. Due to the boosting, clones producing antibodies with a high affinity for the antigen are stimulated selectively.

B cells that secrete antibody specific to the native antigen are then harvested and screened for activity by methods known to those skilled in the art and as described herein. [Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, (1988)]. Some of these screening techniques include, but are not limited to, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay (e.g., using colloidal gold, enzyme or radioisotope labels, for example), western blot, precipitation reactions, agglutination assay (e.g., gel agglutination assays, hemagglutination assays, etc.), complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay, etc.

Once a clone is identified with at least 1:1000 antibody titer, a hybridoma is created by methods known to those skilled in the art and as described herein. [Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, (1988); Kohler and Milstein, *Nature*, 256:495-497 (1975); Kozbor *et al.*, *Immunol. Tod.*, 4:72 (1983); Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. pp.77-96 (1985); PCT/US90/02545; Cote *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:2026-2030 (1983)]. These monoclonal antibodies may then be used for therapies for disease (such as, but not limited to, cancer, viral infections, etc.), immunodiagnosics, immunochemistry, immunohistology, immunocytology, immunoaffinity chromatography, and genomic and proteomic research.

Table 1 provides particular embodiments utilizing the methods of the invention to create high affinity monoclonal antibodies to proteins that traditionally have low immunogenicity. These embodiments are only some examples and are in no way limiting. The first column from the left identifies the antigen of interest. The second column identifies the immunogen that is used to immunize an animal with an intact immune system. In this column, APP refers to the methods described herein wherein the antigen of interest is chemically conjugated to HSP70. Also in this column, KLH conjugate means a conjugate with keyhole limpet hemocyanin rather than HSP70. The third column shows the number of clones produced from the immunization and the fourth column shows the number of clones which are able to bind to the native antigen of interest. The fifth column shows the quantitation method used to identify whether the monoclonal antibodies were specific to the antigen of interest. ELISA is used to identify enzyme linked immunosorbent assay. WB is used to identify western Blot. IF is used to identify immunofluorescence. IAC is used to identify immunoaffinity chromatography. The last column provides a brief explanation for why the antigens do not elicit a strong immune response without conjugation.

TABLE 1
Murine Monoclonal Antibodies Produced
Using Adjuvant Polypeptide (APP) Technology

<i>Target</i>	<i>Immunogen</i>	<i>Total number of clones produced</i>	<i>Number of clones which recognize intact target</i>	<i>Methods</i>	<i>Problem</i>
1. Matrix Metalloproteinase 3 (MMP3), human	Recombinant catalytic domain of MMP-3, 40-212, mol. Mass 19.369 Da – intact	0	0	ELISA	Conservative, low-immunogenic, improper folding
	Recombinant catalytic domain of MMP-3, 40-212, mol. Mass 19.369 Da – APP conjugate	4	1	ELISA, WB	

2. Troponin T, cardio specific, human (TT)	16-mer peptide specific to Troponin T cardiac isoform 1 – KLH conjugate	7	0	ELISA, WB	Multiple isoforms, cross-reactivity
	16-mer peptide specific to Troponin T cardiac isoform 1 – APP conjugate	3	2	ELISA, WB	
3. Erythropoietin, human (EPO)	Recombinant, full size human erythropoietin – intact	0	0	ELISA	Highly conservative, low immunogenic
	Recombinant, full size human erythropoietin – denatured, 6M urea	2	1	ELISA, WB	
	Recombinant, full size human erythropoietin – immune complex with Mab	0	0	ELISA	
	Recombinant, full size human erythropoietin – APP conjugate	5	3	ELISA, WB	
4. Neuron Specific Enolase ($\gamma\gamma$ -subunit), human (NSE)	Natural NSE from human brain, tetramer, mol.mass 160kDa – intact	9	1	ELISA, WB, IF	Conservative, low immunogenic, inactivated by coating
	Natural NSE from human brain, tetramer, mol.mass 160kDa – denatured, 6M urea	0	0	ELISA	

	Natural NSE from human brain, tetramer, mol.mass 160kDa – immune complex with Mab	0	0	ELISA	
	Natural NSE from human brain, tetramer, mol.mass 160kDa – APP conjugate	2	2	ELISA, WB	
5. Tissue Plasminogen Activator, human (TPA)	Recombinant full size human proTPA, mol. Mass 46 KDa – APP conjugate	7	5	ELISA, WB, IAC, Enzymatic activity inhibition	Avoid cross-reactivity with amino-terminal fragment, produce Mab which binds proTPA in 1M GnHCl
6. Parathormone, human (PTH)	N-terminal 1-34 synthetic peptide – APP conjugate	5	5	ELISA	Low immunogenic, small size, expensive need for matched Mab pair.
7. Parathormone, human (PTH)	Mid-molecule 28-48 synthetic peptide – APP conjugate	2	1	ELISA	Low immunogenic, small size, expensive need for matched Mab pair.
8. Parathormone, human (PTH)	Mid-molecule 44-68 synthetic peptide – APP conjugate	2	1	ELISA	Low immunogenic, small size, expensive need for matched Mab pair.

9. Hemo- globin, human (Hb)	Natural Hb from pooled human erythrocytes – intact	0	0	ELISA	Highly conservative, low immuno- genic, need to distinguish glycosylated/ deglycosy- lated isoforms
	Natural Hb from pooled human erythrocytes – denatured, 6M urea	1	1 (glycosy- lated only)	ELISA	
	Natural Hb from pooled human erythrocytes – APP conjugate	3	3 (both)	ELISA	
10. Ghre-lin, human	Full-size 1-28 synthetic peptide, octanoyl-group free – APP conjugate	3	3	ELISA	Small-size, low immuno- genic, conservative
11. Conne- xin 40, human	16-mer peptide specific to human Connexin 40 – KLH conjugate	2	0	ELISA	Low abundance
	16-mer peptide specific to human Connexin 40 – APP conjugate	3	3	ELISA, WB, IF	
12. Inter- feron gamma, human	Recombinant, full-size human interferon gamma, Mol.mass 16.7 kDa – intact	3	2	ELISA	Low-immuno- genic, need to produce neutralizing Mab
	Recombinant, full-size human interferon gamma, Mol.mass 16.7 kDa – APP conjugate	4	3	ELISA	

13. Influenza A virus (H5N1), haemagglutinin	Tentative 16aa and 18 aa peptides type-specific to H5 haemagglutinin 16 AA- APP conjugate	5	2	ELISA, WB	Low abundance, cross-reactivity
	Tentative 16aa and 18 aa peptides type-specific to H5 haemagglutinin 18 AA- APP conjugate	2	1	ELISA, WB	
14. Onco-protein E7 from human Papilloma virus type 18 (E7HPV18)	Recombinant full-size E7HPV18, mol. Mass 11995 Da – intact E7	0	0	ELISA, WB	Immuno-suppression, low-immunogenic, oligomerization, improper folding
	Recombinant full-size E7HPV18, mol. Mass 11995 Da – denatured, 6M urea	0	0	ELISA, WB	
	Recombinant full-size E7HPV18, mol. Mass 11995 Da – recombinant fused protein E7HPV18-APP	3	3	ELISA, WB, IF	
15. Prion Protein human (PrP)	13-mer peptide specific to 130-142 aa of PrP-APP conjugate	5	3	ELISA, WB	Highly conservative, low-immunogenic
16. Clostridium botulinum Neurotoxin A (BoNT/A)	Four peptides specific to H-chain of BoNT/A-APP conjugate	In progress		ELISA, WB	Highly toxic, low immunogenic after inactivation by formaldehyde
17. Hyaluronic acid (HUA)	HUA-APP conjugate	3	3	ELISA	Highly conservative, non-immunogenic

IV. Monoclonal Antibodies for the Detection Human Papilloma Virus/Cervical Cancer

In an embodiment of the invention, the inventive method was used to develop monoclonal antibodies specific to Human Papilloma Virus (“HPV”) E6 and E7 oncoproteins. The development of specific monoclonal antibodies to these oncoproteins have been very difficult because natural E6 and E7 oncoproteins are hard to purify due to their low concentration, and recombinant proteins tend to fold incorrectly and aggregate. Additionally, E6 and E7 oncoproteins have immunosuppressive activity. The antibodies produced using recombinant E6 and E7 oncoproteins as an immunizing agent did not have high affinities for the native E6 and E7 proteins. Because of this, the novel method was employed to create the antibodies, which can be then used for many applications as described herein.

A. Cervical Cancer Background

Cervical carcinoma is the second most prevalent cancer in women. A correlation has been shown between invasive cervical cancer and HPV based on epidemiological and virological studies (**Fig. 2**). A similar correlation has also been found between HPV and neoplasia of the external sex organs and anus, as well as invasive uterine carcinoma [Kiselev, V. I, *et al.*, *Interrelationship of Sexually Transmitted Viral Infections and Oncological Illnesses of the Urogenital Tract In Sexually Transmitted Diseases: Vaccines, Prevention, and Control.* (ed.) D. I. Bernstein. Academic Press, London, *Herald of Dermatology*, No. 6:20-23 (2000)].

Cytological examinations (commonly known as Pap smears) still constitute the principal laboratory diagnostic technique to detect atypical multinucleated cells. This technique has been used for the early diagnosis of cervical cancer for a great many years. These procedures, however, do not detect more than 30% of all HPV carriers and cannot predict the risk of developing a more serious illness.

In studying the structure of HPV deoxyribonucleic acid (“DNA”), including the study of nine immunogenic proteins that are encoded by the DNA, together with the regularities of virus replication, immortalization, and its transformation of epithelial cells, a screening method was developed. The basis of which consisted of detecting biochemical, immunological, and genetic parameters that exhibit deviations from normal values when pathologies are present.

It is not always possible to interpret this type of data to make an early diagnosis, to assess the risk of developing cervical cancer, or to predict the effectiveness of treatment. This is because the measurement techniques used either do not have the requisite sensitivity and/or specificity, the parameters being measured do not directly reflect a cell degeneration process,

or the measurements do not reflect it proportionally.

Techniques have been described for diagnosing cervical cancer by measuring antibody titers specific to HPV proteins in patient sera using enzyme immunoassays (which include, but are not limited to ELISA's) and immunoradiometric assays [*see*, European patents (EPs) No. 386734, 375555, 406542, and 523391]. This approach, however, has not lived up to expectations, since no reliable correlation has been found between an antibody titer and cervical cancer.

Techniques also exist for diagnosing and predicting the development of cervical cancer based on the typing and quantitative determination of the HPV DNA levels in a specimen undergoing study, using the polymerase chain reaction ("PCR"). [V. I. Kiselev, *et al.*, *The Polymerase Chain Reaction During the Diagnosis of Urogenital Infections: A Handbook for Physicians*, Moscow, 2000; World Patent (WO) No. 00506645; and United States (U.S.) Patent No. 5,679,509].

The use of these techniques, however, have lead to considerable over diagnosis, since HPV infection is short-term in nature in roughly 80% of all cases, can culminate in spontaneous recovery, as well as elimination of the virus. *See Fig. 2*. Yet, a positive result for HPV DNA does, in most cases, reliably predict the development of cervical cancer.

The novel solution proposed by the present invention includes a method for the early diagnosis of cervical cancer and the determination of the risk of its development based on the quantitative immunological measurement of HPV E6 or E7 oncoproteins from a biopsic cell sample rather than patient sera [EP No. 1253924, A 61 K 38/18, 08/02/2001].

In a particular embodiment, the E6 and E7 proteins served as HPV markers and were detected in deparaffinized tissue samples via immunofluorescence technique, with digital computer technologies being used (for a quantitative determination) to analyze multidimensional visualized specimens. When the E6 or E7 protein content in biopsic samples exceeded the normal control values by more than 54%, the conclusion was reached that a heightened risk of development of cervical cancer existed. It has also been proposed that other biochemical markers can be used, such as, but not limited to the epidermal growth factor receptor and the insulin-like growth factor.

In yet another embodiment, a screening method was developed that was simpler and less costly that could easily lend itself to standardization due to a high level of sampling and analysis commonality. This screening method has a sensitivity that corresponds to the sensitivity of the immunoflourescence embodiment previously described. This screening method can be used for the early diagnosis of cervical cancer from cytological samples using the HPV E7 oncoprotein as a marker. The ability to use either diagnostic method depends

upon the use of high affinity monoclonal antibodies specific to E6 or E7 oncoproteins. How these antibodies were produced are described herein.

B. Development of Monoclonal Antibodies to E6 and E7

There is a liminal increase in the synthesis HPV E7 protein in reaction to the epithelial cell degeneration process that is preceded by viral DNA integration into cellular DNA. In a particular embodiment, an E7 gene was isolated from human specimens and cloned, then expressed in *Escherichia coli*. Recombinant E7 protein isolated from the bacterial cells was then used to immunize mice. In most cases, however, the antibodies produced by immunization with the recombinant proteins did not interact with the natural protein, since they recognize other determinants. In order to overcome this problem, the method described above was used, wherein the E7 protein was conjugated to HSP70 and then used as the antigen for immunization.

In particular embodiments, two types of highly sensitive monoclonal antibodies against an E7 protein of natural origin were produced, which cross-reacted with the two types of "high-risk" HPVs (HPV16 and HPV18). This cross-reactivity allows detection of 80-85% of all patients with HPV associated neoplasia. The paired use of these two types of monoclonal antibodies, which react to different E7 protein antigenic determinants, enhances the specificity and ensures the absence of a background in diagnostic screens.

C. Screening Methods for HPV

In a particular embodiment, a method for the early and pre-clinical diagnosis of cervical cancer comprises of the quantitative immunological determination of a human papilloma virus (HPV) oncoprotein in a patient biopsic cell sample. The biopsic cell sample is lysed and the cell lysate supernatant is analyzed for HPV E7 protein using monoclonal antibody pairs. In a particular embodiment, the monoclonal antibody pairs belong to the IgG2a and IgG2b subisotypes and they recognize various antigenic determinants of the E7 protein. In a particular embodiment, the IgG2a 716-321, 716-325, 716-332, and 716-343 antibodies bind to at least one E7 antigenic determinant. In another embodiment the IgG2b 716-281 and 716-288 antibodies bind to at least one other E7 antigenic determinant. In another embodiment, one of the two antibody groups is conjugated to an enzyme label as known to those skilled in the art. [See Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, (1988)]. These antibodies are then used in an enzyme linked immunosorbent assay ("ELISA") as well known to those skilled in the art. In a particular embodiment, wherein the E7 protein concentration in a sample is equal to or greater than 0.05 nanograms (ng) per milliliter (ml) a diagnosis of early stage cervical cancer or the risk of its development is positively made. In other particular embodiments, the

quantitative analysis performance conditions were optimized by means of varying the concentration of antibodies for coating polystyrene plates, the conjugate dilution, the buffer solution composition, and the time and temperature of incubation for the ELISAs as well known in the art.

5 In a preferred embodiment, antibody 716-281 specific to one antigenic determinant of the E7 oncoprotein was used for the primary binding of the protein and antibody 715-332 specific to another E7 antigenic determinant was conjugated to an enzyme label. In yet another embodiment, the monoclonal antibodies used are specific to HPV16 E7, but also cross-react with HPV18 E7. HPV16 and HPV18 are the two most prevalent types of “high-
10 risk” HPVs.

In a further embodiment, when a sample is positive for the E7 protein using the ELISA screening method, typing of the HPV is performed. The HPV type may be determined via various methods, including, but not limited to, using polymerase chain reaction, and *in situ* DNA hybridization.

15 V. Monoclonal Antibodies Specific to Prion Protein.

In another embodiment of the invention, the inventive method was used to develop monoclonal antibodies against Prion protein (“PrP”). It is generally believed that a possible cause of spongiform encephalopathy (BSE, mad cow disease) is a conformational transition of a normal Prion protein into a pathogenic isoform. As the pathogenic isoform of PrP
20 accumulates there is subsequent neuronal degradation. Monoclonal antibodies to this protein would be useful as a diagnostic tool for BSE. The main difficulty in producing monoclonal antibodies for this protein is a highly conserved structure of PrP. Multiple immunizations of Balb/c mice with a full-size recombinant bovine PrP both in native and denatured forms did not result in eliciting a sufficient immune response nor the generation of primary positive
25 clones. In order to produce a monoclonal antibody to PrP, a conformational analysis of the PrP molecule was done and three amino acid sequences were selected. These three peptide sequences were from the N-terminal, mid-molecule and C-terminal parts of bovine PrP:

G5: 25-36/62-69 KKRPKPGGGWNT...QPHGGGWG (20 aa) (SEQ ID NOS: 6 and 7, respectively)

30 G3: 109-121 QWNKPSKPKTNIK (13aa) (SEQ ID NO: 8)

G4: 226-242 ITQYQRESQAYYQRGAS (17aa) (SEQ ID NO: 9)

In particular embodiments, these three peptides were chemically conjugated to HSP70 and then used to immunize Balb/c mice using the methods disclosed herein. After completing the immunization schedule, popliteal lymph nodes (other lymph nodes, ascites,
35 blood or spleen may be used as well) were harvested for clones producing antibodies specific

to PrP. Hybridomas were made as well known in the art and as described herein, and hybridomas were screened for production of high affinity monoclonal antibodies. Monoclonal antibodies with high affinity were identified using immunoassays as well known in the art, such as, but not limited to ELISAs. These monoclonal antibodies may then be used
5 for therapies for disease, immunodiagnosics, immunochemistry, immunohistology, immunocytology, immunoaffinity chromatography, and genomic and proteomic research.

VI. Monoclonal Antibodies Specific to Hyaluronic Acid.

In another embodiment of the invention, the inventive method is used to create monoclonal antibodies to hyaluronic acid. Hyaluronic acid is a high molecular weight
10 polymer composed of repeating dimeric units of glucuronic acid and N-acetylglucosamine which forms the core of the complex proteoglycan aggregates found in extracellular matrix. Hyaluronic acid plays a significant role in human connective tissue disorders such as, but not limited to, symptomatic osteoarthritis of the knee joint. Quantitative determination of hyaluronic acid and its degradation products is considered an important diagnostic parameter,
15 which could be achieved with the use of monoclonal antibodies specific to hyaluronic acid.

Due to the non-protein nature of the polymer and its identity in many different species, ranging from bacteria and to mammals, the ability to create the high affinity antibodies of IgG1, IgG2a, IgG2b isotypes is very difficult.

In a particular embodiment, hyaluronic acid was conjugated with HSP70 for
20 immunization and monoclonal antibody production as described for PrP. See Example 9. Monoclonal antibody screening was done using ELISA with the biotinylated hyaluronic acid and streptavidin-horse radish peroxidase as a secondary reagent for the color reaction. This type of ELISA is well known in the art. [Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, (1988)].

25 As a result, three genetically stable hybridoma cell lines were produced, HU.D3 – IgG2a; HU.D9 - IgG1, HU.E3 – IgG3, each secreting high affinity monoclonal antibodies (IgG1 and IgG2a) against hyaluronic acid. These monoclonal antibodies may then be used for therapies for disease, immunodiagnosics, immunochemistry, immunohistology, immunocytology, immunoaffinity chromatography, and genomic and proteomic research.

VII. Monoclonal Antibodies Specific to Matrix Metalloprotease 3

In another embodiment of the invention, the inventive method is used to create monoclonal antibodies to Matrix Metalloprotease 3 (“MMP3.”). Proteins of the matrix metalloprotease (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue
35 remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP’s are

secreted as inactive proproteins, which are activated when cleaved by extracellular proteases. MMP3 is an enzyme which degrades fibronectin, laminin, collagens III, IV, IX, and X, and cartilage proteoglycans. The enzyme is thought to be involved in wound repair, progression of atherosclerosis, and tumor initiation. MMP3 is reported to be implicated in rheumatoid arthritis and some forms of cancer. These monoclonal antibodies may then be used for therapies for disease, immunodiagnostics, immunochemistry, immunohistology, immunocytology, immunoaffinity chromatography, and genomic and proteomic research.

VIII. Kits for Analyzing Risk of Disease Using Monoclonal Antibodies

The present invention also provides diagnostic kits. In some embodiments, the kits are useful for determining whether the subject is at risk of developing a disease or condition, for example, but not limited to, cervical cancer, spongiform encephalopathy, connective tissue disorders, arthritis and metastasis. The nature and use of a kit will depend on the specificity of the monoclonal antibody provided with the kit. The diagnostic kits are produced in a variety of ways. In some embodiments, the kits contain at least one monoclonal antibody that was generated using the methods of the invention for an antigen of traditionally low immunogenicity. In preferred embodiments, the kits contains reagents for conducting an ELISA or other similar immunoassay as well known in the art. In some embodiments, the kit contains instructions for determining whether the subject is at risk for developing the disease or condition. In preferred embodiments, the instructions specify that risk for developing the disease or condition is determined by detecting the presence or absence of the antigen of interest, such as, but not limited to E7 oncoprotein, PrP, MMP3, or hyaluronic acid. In some embodiments, the kits include ancillary reagents such as buffering agents, nucleic acid stabilizing reagents, protein stabilizing reagents, and signal producing systems (*e.g.*, florescence generating systems as Fret systems). The test kit may be packages in any suitable manner, typically with the elements in a single container or various containers as necessary along with a sheet of instructions for carrying out the test. In some embodiments, the kits also preferably include a positive control sample.

EXAMPLES

Example 1

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: M (Molar); μ M (micromolar); mM (millimolar); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μ g (micrograms); ng

(nanograms); l or L (liters); ml (milliliters); μ l (microliters); cm (centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); °C (degrees Centigrade); min. (minutes); sec. (seconds); % (percent); kb (kilobase); bp (base pair); PCR (polymerase chain reaction); aa (amino acid).

5

Example 1

Detection and Typing of Human Papilloma Virus (HPV)

A. Collection of clinical material

Clinical specimens were prepared and transported as well known in the art. A sterile disposable probe was used to take a scrape of clinical specimens of cervical epithelial cells. 10 The probe was washed with 500 μ l of saline (0.83% NaCl) to remove the cells from the probe, with the solution collected into a 1.5ml covered Eppendorf tube. The specimen was then stored until use, usually at 4°C for up to five days and at -10 to -20°C for up to one month.

B. Purification of DNA

15 DNA extraction methods are well known in the art and the methods to purify DNA for the present invention is not limited to this particular example. The specimen is thawed if frozen as well known in the art, and then centrifuged at 10,000 rpm for 1 minute. A pellet is formed at the bottom and the supernatant is discarded. 100 μ l of physiological solution is added to the tube and the pellet is homogenized. 500 μ l of lysis solution is added and 20 vortexed for 10 seconds. The specimen and tube are then incubated at 65°C. The lysis solution contains 6 M Guanidine thiocyanate (Sigma, USA), 10 mM EDTA (Sigma, USA), 1 % Triton X-100 (Sigma, USA), 10 mM Tris HCl, pH 7.3 (Sigma, USA), 1% 2- β -Mercaptoethanol (BioChemical, England). 20 μ l of homogenizing Sorbent (Silica, Sigma, USA) is added and agitated for 10 minutes at 50-100 rpm. The specimen and tube are 25 centrifuged at 10,000 rpm for 1 minute and the supernatant is discarded. 500 μ l of wash solution is added and the tube was vortexed until the pellet has mixed into the solution and centrifuged again at 10,000 rpm for 1 minute, discarding again the supernatant. The wash solution contains 4 M Guanidine thiocyanate (Sigma, USA), 10 mM EDTA (Sigma, USA). 1 ml of 70% ethanol is added to the tube and vortexed well and centrifuged at 10,000 rpm for 1 30 minute discarding the supernatant. This ethanol wash is repeated. The tubes are then opened and the pellet allowed to dry for 5-10 minutes at 55°C. 100 μ l of Nuclease Free Water (Promega, USA) is added to the tube and incubated for 5 minutes at 55°C. Specimens can then be used for polymerase chain reactions. Specimens can be stored at 4°C for up to a week or at -20°C for up to six months.

C. Polymerase Chain Reactions ("PCR") to Type HPV

The PCR reactions were carried out in a 25 µl solution, containing 5 µl of template DNA, 67 mM Tris-HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 100 mM dATP, dCTP, dTTP and dGTP, 10 pmol of each primer and 1 unit of Taq - polymerase (5 units/µl; Promega, USA). As well known in the art, a hot start technique was used. Thermal cycling conditions were: 94°C for 3 minutes, and then 40 cycles at 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 30 seconds. The chart below shows the HPV type and primers used to amplify the DNA of those types of HPV.

Type HPV	Primer 5' – 3'	Amplicon Size (b.p.)
HPV – 16	E7-16-F <i>tga cag ctc aga gga gga g</i> (SEQ ID NO: 10)	118
	E7-16-R <i>gca caa ccg aag cgt aga g</i> (SEQ ID NO: 11)	
HPV - 18	E7-18-F <i>gcg act cag agg aag aaa ac</i> (SEQ ID NO: 12)	195
	E7-18-R <i>ca aag gac agg gtg ttc aga</i> (SEQ ID NO: 13)	

10 The amplification products were analyzed on 1.5% agarose gels and photographed under UV light (254 nm).

Example 2

Designing recombinant plasmids that encode the synthesis of E7 oncoproteins of the HPV16 and HPV18 types.

15 Biopsic tissue samples from the uterine cervixes of patients diagnosed with cervical dysplasia served as the E7 gene source. The clinical specimens were examined for the presence of HPV and were typed using the polymerase chain reaction technique described herein. The E7 genes of the HPV16 and HPV18 types were amplified via the PCR technique using gene-specific primers [the 303–base-pair (bp) fragment for HPV16 and the 324–bp
20 fragment for HPV18] (SEQ ID NOS: 14-17), (*see Fig. 3*) whereupon these genes were cloned at the EcoRI-BamHI sites in a pBluescript SK (+) (Stratagene) plasmid. The determination of the oligonucleotide sequences of the cloned genes demonstrated full agreement with GenBank data (for example, GenBank Accession No.: AF125673 for HPV16 and AY262282 for HPV18). (*See Figs. 4 and 5*, SEQ ID NOS: 1 and 3)

25 Next, translation termination sequences were incorporated into the genes near the 3' ends. The resultant genetic constructs, pHE716 and pHE718, are presented in **Fig. 6** for both

HPV16 and HPV18 (SEQ ID NO: 18).

Synthesis of E7 proteins of the HPV16 and HPV18 types in *E. coli* cells

The plasmid pHE716 encodes the protein E7-16 [Met(His)₆-GluPheIle-E716-GlySer [111 amino-acid residues (“aa”), 12.5 kilodaltons (kDa), and an isoelectric point (IEP) of 4.6]. And the plasmid pHE718 encodes the protein E7-18 [Met(His)₆-GluPheSer-E718-GlySer (117 aa, 13.5 kDa, and an IEP of 5.4). It must be noted that when electrophoresis is performed in sodium dodecyl sulfate and a polyacrylamide gel (“SDS-PAGE”) [Laemmli, U. K., *Nature*, 227:680-685 (1970)] this protein possesses an abnormal mobility (of approximately 21 kDa), which is in agreement with bibliographic data. [Jeon, J.-H., *et al.*, Experimental and Molecular Medicine, 34:496-499,(2002)].

The BL21(DE3) strain of *E. coli* (Stratagen, USA) was used for the expression of the resultant genes. Transfection was performed as well known in the art. Following ultrasonication, HPV16 E7 oncoprotein and HPV18 E7 oncoprotein were detected in a soluble cell protein fraction; therefore, chromatographic analysis on Ni-III A-agarose was performed under native conditions, without the addition of urea (Fig. 7).

More specifically, cells of *E. coli* strain BL21 (DE3) were transformed using the plasmids pHE716 and PHE718. The cells were incubated overnight at 37°C in Luria-Bertani (“LB”) media with the addition of ampicillin (100 µg/ml) and glucose (0.2%). The cell were then diluted 1:25 with fresh LB media with ampicillin and incubated for 2-3 hours until cell density reached an optical density of OD₆₀₀=0.5. Optical density was measured on a Multiscan analyzer at a wavelength of 600 nm. Isopropyl β-D-thiogalactopyranoside (“IPTG”) was then added to a concentration 0.2mM to the culture to a total volume of 50 ml and incubated for additional 3 hours. The cultures were then centrifuged at 5,000 rpm for 10 minutes, with the supernatant discarded. The pellet was resuspended in 1 ml of Tris-HCl 20 mM, pH 8, 0.3 M NaCl 0.1% Triton X-100 and sonicated for 15 seconds. The cultures were then centrifuged at 10,000 rpm for 10 minutes. The pellet was washed with Tris-HCL 20 mM, pH 8, 0.3 M NaCl 0.1% Triton X-100 (0.5 ml). The pellet is homogenized and resuspended in 5 ml of a buffer A (10mM Tris-HCl, pH 8, 6M urea, 0.4 M NaCl, 10mM β-Mercaptoethanol, 5mM Imidazole). The sample is then incubated at room temperature for 1 hour with agitation. The sample is then centrifuged at 10,000 rpm for 10 minutes. The pellet is discarded. A column with IDA-agarose (1 ml) was washed with a solution of NiSO₄, then the column was equilibrated with buffer A. The supernatant was loaded onto the column with 5 ml of buffer A, and then 10 ml of buffer B (Buffer A with 10 mM imidazole). Elution was carried out with Buffer C (10 mM Tris-HCl, pH 8, 2M urea, 0.4 M NaCl, 10mM β-Mercaptoethanol, 0.3M imidazol) in 0.5 ml fractions. Ordinarily, the E7 protein from HPV16

and HPV18 usually eluted in fractions 1-3. The fractions containing the protein were pooled and dialyzed overnight against 100 times the volume of the pooled fractions with a buffer containing 10mM Tris-HCl, pH 8, 150 mM NaCl.

Example 3

5 **Expression of a hybrid protein consisting of E7 peptide of HPV type 16 (18) and DnaK (HSP70) *M. tuberculosis*.**

PCR was used to amplify the E7 gene of human papilloma virus, types 16 and 18. Plasmid DNAs pHE716 and pHE718 containing human papillomavirus (HPV) type 16 and 18 E7 genes were used as a template for PCR amplification of fragments containing E7 genes
10 (SEQ ID NOS: 19-22). *See Fig. 8*. The initiation codon is in frame with the sequence encoding a 6HIS tag, and the stop codon is missing. DnaK protein was expressed using the cloned DNA fragments containing E7 genes transfected into a recombinant pQE30-based vector.

Recombinant vector pQE30-dnaK-Y was previously constructed and used as a basic
15 plasmid, (**Fig. 9**) allowing expression of 6HIS-DnaK fusion protein. The nucleotide sequence of pQE30-dnaK (SEQ ID NO: 5) is shown in **Fig. 10**. To create this vector, PCR fragments containing E7 genes were digested with BamHI and BglII and cloned into the BamHI site of pQE30-dnaK-Y. Recombinants carrying the inserts in correct orientation were identified using restriction analysis as well known in the art. Recombinant plasmids pQE30-E716-dnaK
20 and pQE30-E718-dnaK allowed expression of hybrid proteins 6HIS-E7(type 16)-DnaK and 6HIS-E7(type 18)-DnaK, respectively.

This was accomplished by transfecting *E. coli* DLT1270 cells with pQE30-E716-dnaK and pQE30-E718-dnaK in separate cultures and incubating overnight. (DLT1270 cells were produced by integrating the *lacI* gene into the *E. coli* strain DH10B with the use of P1-transduction. DH 10B has the following genotype : 10 B (ara D139 D (ara, leu) 7697 D (lac)
25 X 74 galU galK rpsL deoR f80 lacZ DM15 end A1 nupG recA1 mcrA D (mrr hsdRMS mcr BC)). *See, Grant, S., et al., Proc. Natl. Acad. Sci. USA, 87:4645-4649 (1990).*) The transfected DLT1270 cultures were grown in Luria-Bertani ("LB") broth that was diluted 100- fold and incubated at 37°C with shaking until a density measured by optical density reached an OD₆₀₀=0.5. Then isopropyl β-D-thiogalactopyranoside ("IPTG") was added up to
30 0.1 mM and the culture was grown as above for extra 2 hours. As well known in the art, IPTG induces the *lacZ* gene and is used to select cells transfected with plasmid of interest. Synthesis of the hybrid proteins of expected molecular weight was observed by SDS-PAGE.

Example 4**Isolation of Recombinant HSP70 Protein from E.Coli****A. Buffer solutions**

5 Buffer solutions are not prepared in advance. Buffer A is prepared before isolation procedure and used within one hour. Stock solution of 50 mM K₂HPO₄, pH 7 may be prepared in advance but must be used within three days. 10X PBS stock solution may also be prepared in advance. Storage of buffer solutions, and chromatographic procedures should be conducted at room temperature.

10 Buffer A comprises 25 mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) ("HEPES"), pH7.3, 0.1% Nonidet P-40 (w/v), 20% glycerol (v/v), 1 mM Phenylmethylsulphonyl fluoride ("PMSF"), and 0.5 M NaCl.

Buffer B comprises 50 mM K₂HPO₄, pH 7, 5 mM Imidazole, 0.5 M NaCl, pH is not adjusted.

Buffer C comprises 50 mM K₂HPO₄, pH 7, 150 mM Imidazole, 0.5 M NaCl.

15 Buffer D comprises 50 mM K₂HPO₄, pH 7, 50 mM EDTA, 0.5 M NaCl, pH is not adjusted.

PBS buffer comprises 10 mM K₂HPO₄, pH 7.5 and 0.145 M NaCl.

B. Preparation of Chelating Column

20 Wash the 1 x 10 cm column with de-ionized water, forcing the bubbles from the lower filter, leaving a layer of water at the level of approximately 1 cm from the lower filter. Measure the necessary amount of Chelating Sepharose CL-6B (Amersham Pharmacia Biotech) to make a volume of 5ml. Using a pipette load the column with the Sepharose gel. Wash the column with 5 volumes of de-ionized water at the rate of 3 ml/minute. Then wash the column with 10 volumes of solution ZnCl₂ (concentration of 1 mg/ml (ICN)) at a rate of 3 ml/minute. Then wash the column with 10 volumes of de-ionized water at a rate of 3 ml/minute. Connect the column to a UV detector (280nm). Then wash the column with 10 volumes of water at a rate of 3 ml/ minute. The column is now prepared for isolation of protein from *E. coli* cells grown in 500 ml of culture medium.

D. Preparation of *E. coli*

30 Procedures are to be conducted at 4°C as quickly as possible. First, wash the 1 gram *E. coli* (wet biomass) with 2 volumes of PBS 5 times by centrifuging at 5000 rpm for 15 seconds each cycle and then resuspending bacterial cells. Dilute cells after final wash in 5 ml PBS. The cells are then sonicated (20 kHz, 50-60 W) 6 times for 30 seconds. The resulting solution is centrifuged at 12,000 rpm for 10 minutes. The pellet is discarded, and the
35 supernatant is loaded onto to the column.

E. Separation of Protein

After the supernatant has been loaded, the column is washed with the column 10 volumes of Buffer A. This is followed by 5 volumes of Buffer B. Protein fractions are eluted with 5 ml Buffer C and collected.

5 F. Dialysis of the protein recovered from the column

The pooled fractions are placed in a dialysis bag and is dialyzed for 30 minutes against 200 volumes of PBS at 0°C with constant stirring. After 30 minutes the solution is replaced with a fresh 200 volumes of PBS at 0°C also with constant stirring. The fractions are then dialyzed over night in 5 liters of PBS at 40°C. Determine the protein concentration by
10 Bradford or Lowry protein quantitation methods as well known in the art. [Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, (1988)]. Freeze the solution at -700°C and lyophilize.

Example 5

15 **Production of mouse monoclonal antibodies against recombinant HPV16 and HPV18 E7 proteins**

Balb/c mice [10 females weighing 16-18 grams (g)] were immunized twice in the hind foot pads, with a two-week interval between immunizations, using highly purified HPV16 E7 and HPV18 E7. The HPV16 E7 and HPV18 E7 preparations of recombinant *E. coli* lysates were purified using single-stage metal-chelate chromatography. The first
20 immunization contained 20 µg of protein diluted in 20 µl of phosphate buffered saline ("PBS") mixed with an equal volume of Freund's complete adjuvant. The second immunization also contained 20 µg of protein diluted in 20 µl of PBS, but was mixed with an equal volume of Freund's incomplete adjuvant. On the fourth day after the second immunization, lymphocytes from the popliteal lymph nodes were hybridized to Sp2/0-Ag14
25 myeloma cells (American Type Culture Collection ("ATCC") CRL 1581; ATCC CRL 8287; European Collection of Cell Cultures 85072401; DSMZ Human and Animal Cell Cultures ACC 146) using polyethylene glycol (PEG) 4000. The hybridomas were incubated in hypoxanthine-aminopterin-thymidine ("HAT") medium two weeks at 37°C with 5% CO₂. Surviving selected hybridomas were screened using an indirect ELISA. The positive cultures
30 were cloned twice using the limited dilution technique. [See Campbell, A. M., *Monoclonal Antibody and Immunosensor Technology in Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 23 (1991)]. The ELISA was performed by absorbing the recombinant HPV16 and HPV18 E7 proteins onto polystyrene plates at a concentration of 2 µg/ml by incubating the plates for 1 hour at 37°C, the plates were then washed 3 times with PBS, 50 µl
35 of the tissue culture supernatants was added to the plates and incubated for 1 hour at 37°C.

The monoclonal antibodies that bound to the immobilized antigen were detected using goat anti-mouse immunoglobulin G (IgG) [H+L] conjugated to peroxidase for a period of 1 hour at 37°C. A tetramethylbenzidine ("TMB") solution that contained hydrogen peroxide served as the substrate. Optical density was measured at 450 nm. Two groups of hybridomas were obtained for the HPV16 E7 protein that produced 716-281 and 716-288 (IgG2b) antibodies, as well as 716-321, 716-325, 716-332, and 716-343 (IgG2a) antibodies, which interacted equally well with HPV16 and HPV18 E7 in an indirect ELISA (*see* Table 2).

TABLE 2
Optical density values at 450 nm in an indirect ELISA
during monoclonal antibody titration
using immobilized HPV16 and HPV18 E7 proteins

		MoAb concentration, in ng/ml					
		100	30	10	3	1	0
HPV16 E7 coating	716-281	>2	1.777	0.947	0.398	0.154	0.017
	716-288	>2	1.515	0.705	0.301	0.091	0.007
	716-321	>2	1.382	0.462	0.160	0.047	0.013
	716-325	>2	1.131	0.375	0.117	0.030	-0.008
	716-332	>2	1.393	0.473	0.168	0.045	-0.012
	716-343	>2	1.737	0.652	0.265	0.003	0.0
HPV18 E7 coating	716-281	>2	1.807	1.010	0.445	0.176	0.029
	716-288	>2	1.709	0.939	0.412	0.170	0.026
	716-321	>2	>2	1.331	0.508	0.176	0.021
	716-325	>2	>2	1.194	0.425	0.145	0.020
	716-332	>2	>2	1.350	0.526	0.190	0.025
	716-343	>2	>2	1.672	0.839	0.365	0.028

Example 6

Optimization of enzyme immunoassay performance conditions for the quantitative determination of HPV16 and HPV18 E7 proteins using monoclonal antibodies

Monoclonal antibodies against HPV16 E7 were collected from mouse ascites and purified on a protein G-Sepharose affinity column (a purity of more than 95%). The antibodies were labeled with horseradish peroxidase using the periodate technique. All the paired monoclonal antibody combinations were checked for antigen affinity for HPV16 E7 by ELISA. To perform the monoclonal antibody pairing, one member of the monoclonal

antibody pairs was immobilized on a polystyrene plate. Seven twofold dilutions of HPV16 E7 protein spanning a concentration range of 0.039-5.0 ng/ml were introduced to the monoclonal antibody immobilized on the tray. Immune complexes formed were detected by adding the second member of the monoclonal antibody pair conjugated to peroxidase with tetramethylbenzidine serving as the substrate. All of the monoclonal antibody pairs tested had the ability to form a ternary complex (a sandwich) – an immobilized antibody-E7-conjugated antibody– which suggests the oligomeric state of the HPV16 E7 protein in a solution, even at a concentration range of 10^{-12} - 10^{-9} moles (M). Despite the fact that all the monoclonal antibody combinations worked, pairs of identical antibodies, or antibodies that belonged to the same immunoglobulin isotype exhibited a lower level of sensitivity (the optical density value associated with a fixed HPV16 E7 concentration and the calibration curve slope) than pairs of monoclonal antibody from different isotypes. In terms of sensitivity and the absence of a background, the best results were obtained when 716-281 monoclonal antibodies were bound to the plate and 716-332 monoclonal antibodies were used as the peroxidase conjugate. The assay using this monoclonal antibody pair was optimized by varying the concentration of the antibodies for absorption, the amount of conjugate dilution, the buffer solution composition, and the time and temperature for all stages of the ELISA. A calibration curve for the quantitative determination of HPV16 E7 under optimum conditions is presented in **Fig. 11**. The 716-288 monoclonal antibodies were absorbed from a 0.1 molar (M) carbonate buffer solution with a pH of 9.6 and a concentration of 5 µg/ml. The working dilution of the peroxidase-containing 716-332 MoAb conjugate was 1/5,000 in phosphate-buffered saline (PBS) that contained 0.2% bovine serum albumin (BSA) and 0.05% Tween 20. The substrate was tetramethylbenzidine (TMB). The protein concentration is shown along the *x*-axis, in ng/ml. Optical density at 45 nanometers (nm) is shown along the *y*-axis. The experimental error for three independent measurements is shown along this curve. As is apparent from the figure, the calibration curve is almost linear over an antigen concentration range of 0.039-5.0 ng/ml, and is also characterized by the total absence of a background. The presence of HPV16 E7 can be detected with as little as 40 picograms (pg) per ml, which is sufficient for determining the level of HPV16 E7 expression in transfected cells and clinical specimens (*see Fig. 11*).

Example 7

Measurement of the E7 oncoprotein in cervical samples

A specimen from a uterine cervix scraping was placed into 1 ml of saline (0.83% NaCl) and frozen and thawed three times. The sample was then microcentrifuged (Eppendorf) for 10 minutes at 10,000 rpm. The supernatant was diluted 1:1 (v/v) in PBS-AT

(PBS that contains 0.2% BSA and 0.1% Tween), whereupon 200 μ l was introduced into the well of a plate previously bound by anti-HPV16 E7 monoclonal antibodies at a concentration of 5 μ g/ml and was titrated with a two-fold serial dilution at 4 wells. Within this same plate, a purified recombinant HPV16 E7 protein was titrated from 4 ng/ml to 0.062 ng/ml as the standard. After one hour of incubation and washing, a peroxidase-labeled conjugated anti-HPV16 E7 monoclonal antibodies were introduced, incubation was continued for 1 hour, and a TMB reaction was developed. Optical density was measured on a Multiscan analyzer at a wavelength of 450 nm. A calibration curve was plotted based on the optical density (OD) values of the standard's dilutions, by means of which the E7 concentration in a sample was determined.

Screening studies were conducted ($n = 100$). Specimens from healthy patients among whom HPV was not detected, or among whom infection by "low-risk" HPV was detected, served as the control ($n = 10$).

The samples from all the healthy patients were negative for E7, or were slightly positive in certain cases. But, the E7 content was less than 0.05 ng/ml in all cases.

The positive samples exceeded the concentration threshold of 0.05 ng/ml. The data obtained are presented in Table 2.

TABLE 2
Determination of the E7 oncoprotein in cervical samples

Patient No.	Anti-E7 IgG	E7-16	Total protein OD 1/20	PCR confirmation	Diagnosis
P1	0.678 0.418	0.1 ng/ml	0.084	HPV 16 and 18	CIN* III
P2	0.466 0.317	2.2 ng/ml	0.151	HPV 31	CIN I-II
P3	0.232 0.127	0.8 ng/ml	0.152	HPV with a high oncogenic risk	CIN II-III
P4	0.337 0.158	3.5 ng/ml	0.228		CIN III and suspicion of cervical carcinoma
P5	0.268	10 ng/ml	0.041	HPV 16 and 18	CIN III and suspicion of cervical carcinoma
P6	0.422 0.202	1 ng/ml	0.058	HPV 16 and 18	CIN II
P7	0.215	0.06 ng/ml	0.174	HPV 16 and 18	CIN II-III

P8	0.357 0.164	0.3 ng/ml	0.089	HPV 16	CIN I-II
P9	0.412 0.197	1.6 ng/ml	0.096	HPV 18	CIN II-III
P10	0.335 0.130	0.9 ng/ml	0.165	HPV 16	CIN III and suspicion of cervical carcinoma
P11	0.280	1.2 ng/ml	0.180	HPV 16	CIN II
P12	0.554 0.233	0.8 ng/ml	0.220	HPV 16	CIN II-III
P13	0.443	0.8 ng/ml	0.097	HPV 16	CIN I-II
P14	0.214	1.8 ng/ml	0.162	HPV 16	CIN II

*CIN—cervical intraepithelial neoplasia

As shown by the data, the results of the diagnostic screening method correspond with the medical diagnosis. The HPV typing data also correspond to the diagnostic data and can thereby be used to predict the risk of development of cervical cancer.

5

Example 8

Production of Monoclonal Antibodies to PrP.

Each peptide, G3, G4 and G5, was synthetically produced as known to those skilled in the art. [*Fmoc Solid Phase Peptide Synthesis, A Practical Approach*, eds. W.C. Chan and P.D. White, in *The Practical Approach Series*, series ed. B.D. Hames, Oxford University Press, (2000).] The peptides were lyophilized, and tested to be essentially salt-free, and 95% pure by HPLC. One hundred micrograms of each peptide was reconstituted with 0.1 ml 100 mM sodium phosphate buffer, pH 6.8 (coupling buffer) to create a "peptide solution". A solution of HSP70 (1mg/ml) was made with coupling buffer to make a "HSP70 solution". For each peptide, peptide solution (0.1 ml) was mixed with 0.2 ml HSP70 solution, containing 200 ug of the protein, yielding approximately 15-20 fold molar excess of the peptide over HSP70. Glutaraldehyde solution (25% glutaraldehyde with water) was added immediately up to 0.05% (v/v) to the mixture and incubated for 3 hours at room temperature with constant shaking. The coupling reaction was stopped by adding 0.5 M glycine solution to 0.1 M final concentration and incubated for another 30 minutes under the same conditions. The reaction mixture was then dialyzed against 100 ml PBS (10 mM Sodium Phosphate, 150 mM Sodium Chloride, pH 7.2) at 4°C overnight. The efficiency of the coupling reaction was checked by SDS-PAGE as well known in the art and results showed that there was no free HSP70 remaining. The conjugate produced was aliquoted and stored at -70°C without preservatives added.

25

Female Balb/c mice (18-20 g) were immunized twice into the hind footpads with 50

µl of the peptide-hsp70 conjugate solution with a two-week interval in between. At day 16 popliteal lymph nodes were harvested and processed for B cells for fusion with appropriate Balb/c myeloma cell line using conventional hybridoma technology as well known in the art and described herein.

5 The primary screening was done by indirect ELISA using G3, G4, G5 peptides, coated onto polystyrene plates with the following results:

Peptide G3 – hybridization yielded a small number of primary cultures, with even less number of positive ones, which later lost their activity.

Peptide G4 – 4 clones from different groups were established, thrice cloned.

10 Peptide G5 - 6 clones from different groups were established, thrice cloned.

Ascites were produced and also tested on corresponding peptides and full-size recombinant PrP. Table 3 shows the name of the clones isolated, the peptide each is specific to, and the immunoglobulin isotype. Table 4 shows the results of an indirect ELISA wherein the peptides used for making the conjugated protein is absorbed onto the polystyrene plate.

15 Table 5 shows the results of an indirect ELISA wherein the native PrP protein is absorbed onto the polystyrene plate

TABLE 3

Immunogen and mouse Immunoglobulin sub-isotype specification of the Mab produced

Clone	Immunogen	Isotype
4G11	G4-hsp70	IgG2b
4G29	G4-hsp70	IgG2b
4G57	G4-hsp70	IgG2b
4G69	G4-hsp70	IgG2a
5G13	G5-hsp70	IgG3
5G29	G5-hsp70	IgG1
5G38	G5-hsp70	IgG3
5G48	G5-hsp70	IgG1
5G55	G5-hsp70	IgG1
5G77	G5-hsp70	IgG1

TABLE 4
Monoclonal antibody indirect ELISA titration data (OD450 nm) using peptides for coating

Coating Antigen	Peptide G4					Peptide G5				
	4G11	4G29	4G57	4G69	5G13	5G29	5G38	5G48	5G55	5G77
1/1000	1.428	1.527	1.479	*	*	*	*	*	1.604	1.643
1/3000	1.267	1.456	1.279	*	1.815	*	*	1.880	1.629	1.866
1/9000	1.016	1.412	1.094	*	1.824	*	*	1.838	1.572	*
1/27000	0.621	1.176	0.644	*	1.659	1.863	1.815	1.793	1.530	1.882
1/81000	0.284	0.877	0.273	*	1.306	1.691	1.018	1.613	1.421	1.811
1/243000	0.107	0.392	0.071	1.651	0.719	1.255	0.388	1.375	1.216	1.502
1/729000	0.035	0.118	0.010	0.755	0.247	0.725	0.114	0.752	0.873	0.961
1/2187000	0.015	0.043	0.003	0.270	0.044	0.330	0.039	0.294	0.481	0.537

TABLE 5
Monoclonal antibodies indirect ELISA titration data (OD450 nm) using PrP for coating

Coating Antigen	Prion protein										
	4G11	4G29	4G57	4G69	5G13	5G29	5G38	5G48	5G55	5G77	
Ascite Dilution											
1/1000	0.139	0.089	0.137	0.257	0.193	0.296	0.138	0.129	1.832	0.159	
1/3000	0.056	0.039	0.063	0.109	0.070	0.129	0.035	0.041	1.646	0.109	
1/9000	0.036	0.031	0.044	0.070	0.030	0.093	0.014	0.018	1.726	0.088	
1/27000	0.030	0.027	0.034	0.045	0.018	0.066	0.002	0.005	1.739	0.050	
1/81000	0.027	0.025	0.030	0.027	0.005	0.033	0	0.004	1.742	0.032	
1/243000	0.026	0.079	0.026	0.025	0	0.014	0	0.003	1.745	0.014	
1/729000	0.025	0.023	0.025	0.026	0	0.009	0	0.001	1.550	0.003	
1/2187000	0.031	0.024	0.024	0.024	0	0.001	0	0	1.168	0.002	

All of the monoclonal antibodies shown above have high affinity for the immunogens used in generating them. In one particular embodiment, however, monoclonal antibody 5G55 strongly reacts with recombinant PrP. This antibody can be used for any applications utilizing ELISA, western blot and immunohistochemistry. The other remaining monoclonal antibodies show low to moderate binding specificity to recombinant PrP.

Example 9

Production of Monoclonal Antibodies to MMP 3.

As previously described for other antigens, BALB/c mice were immunized in their hind foot pads with MMP 3 (25µg/mouse). The mice received a second immunization 2 weeks later. On the fourth day after second immunization, lymph nodes were harvested and B cells from the nodes were fused with myeloma Sp2/0-Ag14 cells and placed into the 96-well plates. The immune sera was also collected and tested.

Table 6 shows the results of an indirect ELISA measured at OD 450 nm using MMP3 at 2 µg/ml in PBS that was absorbed onto polystyrene plates at 4°C overnight. All other incubations were performed in PBS with 0.5%BSA and Tween 20 as well known in the art. The results show that serum from mice immunized by MMP3 did not show any significant difference from serum of non-immune mice.

TABLE 6

Indirect ELISA measured at OD 450 nm using MMP3--Unconjugated

Serum Dilution	OD₄₅₀ Meas.
Immune serum	
1:1000	0.52
1:3000	0.42
1:9000	0.36
1:27000	0.37
Nonimmune serum	
1:1000	0.53
1:3000	0.31
1:9000	0.25
1:27000	0.30

All primary clones were also tested by indirect ELISA on the MMP3 and by direct ELISA with biotin-labeled MMP3. No positive clones were created using unconjugated MMP3. MMP3 was then conjugated to HSP70 using the same method as described above for PrP using glutaraldehyde.

Conjugated MMP3 (50µg/mouse) was then used to immunize Balb/c mice as previously described. On the fourth day after the second immunization, lymph nodes were

harvested and processed as well known in the art. Isolated B cells were fused with myeloma sp 2/0 cells and placed into the 96-well plates. The immune sera were also collected and tested. Results are shown on Table 7. Table 7 shows the results of an indirect ELISA measured at OD 450 nm using MMP3 2 µg/ml PBS that was absorbed onto polystyrene plates at 4°C overnight. All other incubations were performed in PBS with 0.5% bovine serum albumin (“BSA”) and Tween 20 as well known in the art. The results show that serum from mice immunized with conjugated MMP3-HSP70 has significant affinity for MMP3 compared to serum of non-immune mice.

TABLE 7

10 **Indirect ELISA measured at OD 450 nm using MMP3--Conjugated**

Serum Dilution	OD₄₅₀ Meas.
Immune serum	
1:1000	1.252
1:3000	0.835
1:9000	0.596
1:27000	0.452
Nonimmune serum	
1:1000	0.258
1:3000	0.268
1:9000	0.279
1:27000	0.277

All primary clones were also tested by indirect ELISA using MMP3 (2µg/ml) and direct ELISA with biotin-labeled MMP3 (500ng/ml). Eight positive clones were detected by indirect ELISA and 6 positive clones were detected by direct ELISA. All positive clones were cloned, four of them had stabile antibody production and were cloned 2-3 times. Table 8 identifies the clones and immunoglobulin isotypes. Table 9 shows the results of an indirect ELISA using 1 µg/ml of MMP3 absorbed onto the plate and direct ELISA using 0.5 µg/ml MMP3-biotin. The results show that the clones have a high affinity for MMP3.

TABLE 8

20 **Monoclonal antibody producing clones for MMP3 and immunoglobulin isotypes**

Name of clone	Antibody isotypes
MMP3H2	IgG3
MMP3H10	IgG3
MMP3G7	IgG2a
MMP3F11	G3

TABLE 9
Characteristics of the antibodies against MMP3

MoAb, ng/ml	MMP3 G7, Indirect ELISA	MMP3 G7, Direct ELISA	MMP3 F11, Indirect ELISA	MMP3 F11, Direct ELISA	MMP3 H2, Indirect ELISA	MMP3 H2, Direct ELISA	MMP3 H10, Indirect ELISA	MMP3 H10 Direct ELISA
10 000	****	0,093	0,772	0,474	****	0,057	****	0,102
3 000	1,765	0,020	0,295	0,472	1,824	0,090	1,809	0,055
1 000	1,795	0,023	0,182	0,384	1,782	0,126	1,829	0,077
300	1,830	0,037	0,044	0,269	1,607	0,088	1,793	0,025
100	1,745	0,009	0,021	0,147	1,248	0,038	1,683	0,017
30	1,470	0,000	0,015	0,069	0,719	0,033	1,391	0,019
10	0,957	0,000	0,000	0,057	0,295	0,003	0,941	0,004
3	0,498	0,000	0,000	0,051	0,099	0,000	0,463	0,005

All publications and patents mentioned in the above specification are herein
5 incorporated by reference. Various modifications and variations of the described method and
system of the invention will be apparent to those skilled in the art without departing from the
scope and spirit of the invention. Although the invention has been described in connection
with specific preferred embodiments, it should be understood that the invention as claimed
should not be unduly limited to such specific embodiments. Indeed, various modifications of
10 the described modes for carrying out the invention, which are obvious to those skilled in
immunology, medical biochemistry, molecular biology, or related fields, are intended to be
within the scope of the following claims.

We Claim:

1. A method of producing monoclonal antibodies specific to an antigen of low immunogenicity comprising:
 - a. conjugating the antigen chemically to a carrier molecule;
 - 5 b. immunizing an animal with the conjugated antigen;
 - c. harvesting B cells from the animal;
 - d. creating a hybridoma from the harvested B cells;
 - e. screening the hybridomas for specificity to the native antigen.
2. The method of claim 1, wherein the carrier molecule is HSP70.
- 10 3. The method of claim 1, wherein the animal has an intact immune system.
4. The method of claim 1, wherein the animal is a mammal.
5. The method of claim 1, wherein the B cells are harvested from ascites.
6. The method of claim 1, wherein the B cells are harvested from lymph nodes.
7. The method of claim 1, wherein the B cells are harvested from blood.
- 15 8. The method of claim 1, wherein the B cells are harvested from spleen.
9. The method of claim 1, wherein the hybridoma is created using an immortal mouse cell.
10. The method of claim 9, wherein the immortal mouse cell is a mouse myeloma cell.
11. The method of claim 1, wherein the hybridoma is created using an immortal human
20 cell.
12. The method of claim 1, wherein the hybridoma is created using an immortal rat cell.
13. The method of claim 1, wherein the screening for specificity is done by a method
chosen from the group consisting of radioimmunoassay, enzyme-linked
25 immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel
diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western
blot, precipitation reaction, agglutination assay, complement fixation assay,
immunofluorescence assay, protein A assay, virus visualization assay, biological
activity modulation assay, and immunoelectrophoresis assay.
14. A composition comprising a monoclonal antibody specific to an antigen of low
30 immunogenicity produced by:
 - a. conjugating the antigen chemically to a carrier molecule;
 - b. immunizing an animal with the conjugated antigen;
 - c. harvesting B cells from the animal;
 - d. creating a hybridoma from the harvested B cells; and
 - 35 e. screening the hybridomas for specificity to the native antigen.

15. The composition of claim 14, wherein the carrier molecule is HSP70.
16. The composition of claim 14, wherein the animal has an intact immune system
17. The composition of claim 14, wherein the animal is a mammal.
18. The composition of claim 14, wherein the B cells are harvested from ascites.
- 5 19. The composition of claim 14, wherein the B cells are harvested from lymph nodes.
20. The composition of claim 14, wherein the B cells are harvested from blood.
21. The composition of claim 14, wherein the B cells are harvested from spleen.
22. The composition of claim 14, wherein the hybridoma is created using mouse myeloma cells.
- 10 23. The composition of claim 14, wherein the hybridoma is created using an immortal human cell.
24. The composition of claim 14, wherein the hybridoma is created using an immortal rat cell.
25. The composition of claim 14, wherein the screening for specificity is done by a method
15 chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological
20 activity modulation assay, and immunoelectrophoresis assay.
26. A method of producing monoclonal antibodies specific to E7 oncoprotein comprising:
- a. conjugating the E7 oncoprotein chemically to a carrier molecule;
 - b. immunizing an animal with the conjugated antigen;
 - c. harvesting B cells from the animal;
 - 25 d. creating a hybridoma from the harvested B cells; and
 - e. screening the hybridomas for specificity to the native E7 oncoprotein.
27. The method of claim 26, wherein the chemical conjugation comprises:
- a. creating a plasmid with an nucleotide sequence encoding E7 oncoprotein and an nucleotide sequence encoding HSP70; and
 - 30 b. transfecting a host cell with the plasmid, wherein the host cell transcribes the nucleotide sequences into the conjugated E7 oncoprotein.
28. The method of claim 27, wherein the nucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 1.
29. The method of claim 27, wherein the nucleotide sequence encoding E7 oncoprotein is
35 SEQ ID NO: 3.

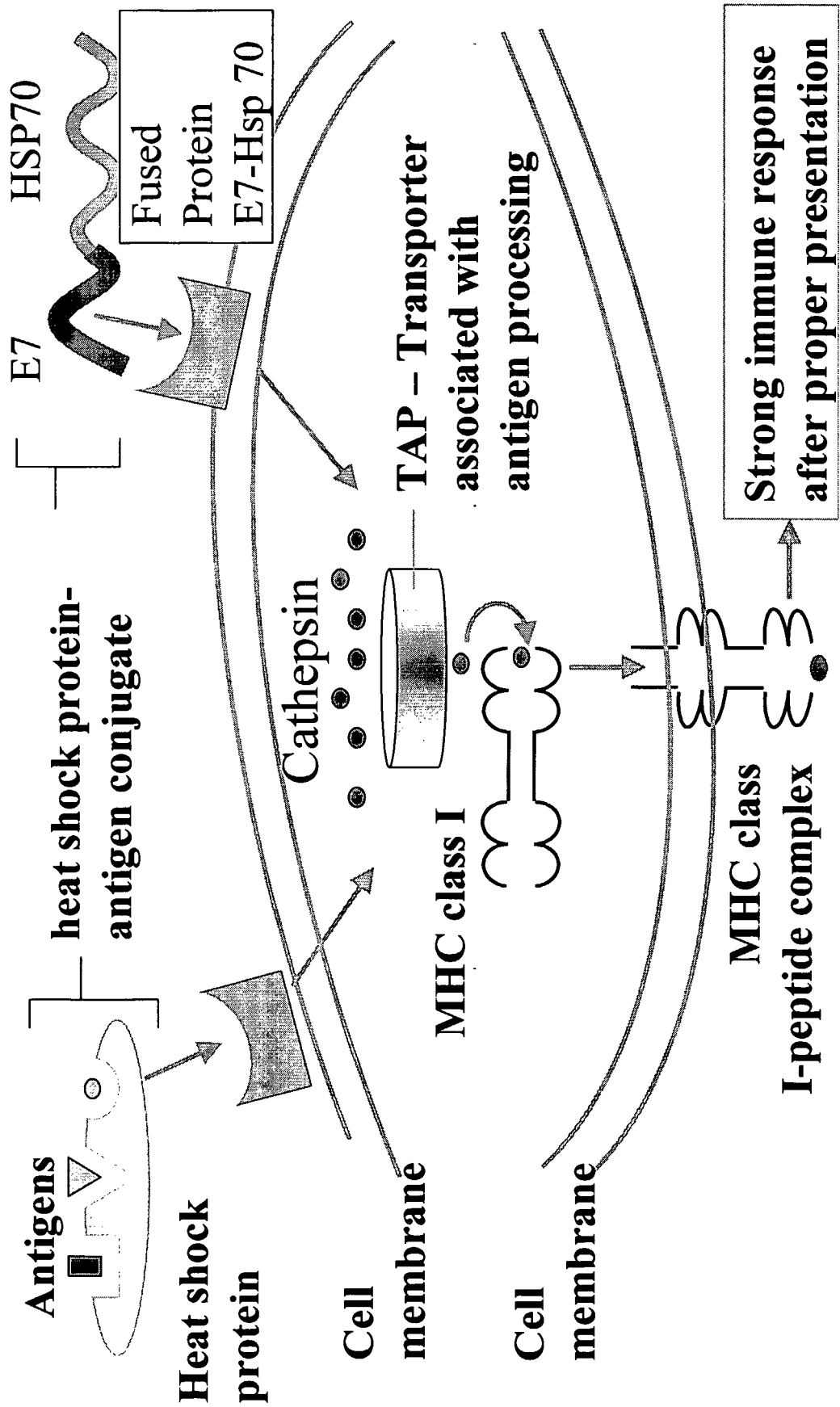
30. The method of claim 27, wherein the nucleotide sequence encoding HSP70 is SEQ ID NO: 5.
31. The method of claim of claim 27, wherein the host cell is *E. coli*.
32. The method of claim 26, wherein the carrier molecule is HSP70.
- 5 33. The method of claim 26, wherein the animal has an intact immune system
34. The method of claim 26, wherein the animal is a mammal.
35. The method claim 34, wherein the animal is a mouse.
36. The method of claim 26, wherein the B cells are harvested from ascites.
37. The method of claim 26, wherein the B cells are harvested from lymph nodes.
- 10 38. The method of claim 26, wherein the B cells are harvested from blood.
39. The method of claim 26, wherein the B cells are harvested from spleen.
40. The method of claim 26, wherein the hybridoma is created using an immortal mouse cell.
41. The method of claim 40, wherein the immortal mouse cell is a mouse myeloma cell.
- 15 42. The mouse myeloma cell of claim 41 is an Sp2/0-Ag14 myeloma cell.
43. The method of claim 26, wherein the hybridoma is created using an immortal human cell.
44. The method of claim 26, wherein the hybridoma is created using an immortal rat cell.
45. The method of claim 26, wherein the screening for specificity is done by a method
- 20 chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.
- 25 46. A composition comprising monoclonal antibodies specific to E7 oncoprotein produced by a method comprising:
- a. conjugating the E7 oncoprotein chemically to a carrier molecule;
 - b. immunizing an animal with the conjugated antigen;
 - 30 c. harvesting B cells from the animal;
 - d. creating a hybridoma from the harvested B cells; and
 - e. screening the hybridomas for specificity to the native E7 oncoprotein.
47. The composition of claim 46, wherein the chemical conjugation comprises:
- a. creating a plasmid with an nucleotide sequence encoding E7 oncoprotein and an
 - 35 nucleotide sequence encoding HSP70; and

- b. transfecting a host cell with the plasmid, wherein the host cell transcribes the nucleotide sequences into the conjugated E7 oncoprotein.
48. The composition of claim 47, wherein the nucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 1.
- 5 49. The composition of claim 47, wherein the nucleotide sequence encoding E7 oncoprotein is SEQ ID NO: 3.
50. The composition of claim 47, wherein the nucleotide sequence encoding HSP70 is SEQ ID NO: 5.
51. The composition of claim 47, wherein the host cell is *E. coli*.
- 10 52. The composition of claim 46, wherein the carrier molecule is HSP70.
53. The composition of claim 46, wherein the animal has an intact immune system
54. The composition of claim 46, wherein the animal is a mammal.
55. The composition claim 54, wherein the animal is a mouse.
56. The composition of claim 46, wherein the B cells are harvested from ascites.
- 15 57. The composition of claim 46, wherein the B cells are harvested from lymph nodes.
58. The composition of claim 46, wherein the B cells are harvested from blood.
59. The composition of claim 46, wherein the B cells are harvested from spleen.
60. The composition of claim 46, wherein the hybridoma is created using an immortal mouse cell.
- 20 61. The composition of claim 60, wherein the immortal mouse cell is a mouse myeloma cell.
62. The mouse myeloma cell of claim 61 is an Sp2/0-Ag14 myeloma cell.
63. The composition of claim 46, wherein the hybridoma is created using an immortal human cell.
- 25 64. The composition of claim 46, wherein the hybridoma is created using an immortal rat cell.
65. The composition of claim 46, wherein the screening for specificity is done by a method chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel
- 30 diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.
66. A method of using monoclonal antibodies specific to E7 oncoprotein for the detection
- 35 of cervical intraepithelial neoplasia comprising:

- a. obtaining a specimen of cervical epithelial cells; and
 - b. screening the specimen for the presence of E7 oncoprotein.
67. The method of claim 66, wherein the screening method for the presence of E7 oncoprotein is chosen from the group consisting of radioimmunoassay, enzyme-linked immunosorbant assay, "sandwich" immunoassay, immunoradiometric assay, gel diffusion precipitation reaction, immunodiffusion assay, *in situ* immunoassay, western blot, precipitation reaction, agglutination assay, complement fixation assay, immunofluorescence assay, protein A assay, virus visualization assay, biological activity modulation assay, and immunoelectrophoresis assay.
68. The method of claim 66, wherein the presence of E7 oncoprotein is equal to or greater than 0.05 ng/ml.
69. The method claim 66, wherein the monoclonal antibodies comprise of at least two immunoglobulin isotypes.
70. The monoclonal antibodies of claim 69, wherein one immunoglobulin isotype is IgG2a.
71. The monoclonal antibodies of claim 69, wherein one immunoglobulin isotype is IgG2b.
72. The monoclonal antibodies of claim 69, wherein one immunoglobulin isotype has specificity for a different antigenic determinant than the second immunoglobulin isotype.
73. A kit for determining if a subject is at risk for developing cervical intraepithelial neoplasia comprising:
- a. at least one reagent that specifically detects E7 oncoprotein; and
 - b. instructions for determining that the subject is at increased risk of developing cervical intraepithelial neoplasia.
74. The reagent of claim 73 is the monoclonal antibodies of claim 46.
75. A method of producing monoclonal antibodies specific to a Prion protein peptide comprising:
- a. conjugating the Prion protein peptide chemically to a carrier molecule;
 - b. immunizing an animal with the conjugated antigen;
 - c. harvesting B cells from the animal;
 - d. creating a hybridoma from the harvested B cells; and
 - e. screening the hybridomas for specificity to the native Prion protein.
76. The method of claim 75, wherein the conjugating is performed chemically using glutaraldehyde.

77. The method of claim 75, wherein the Prion protein peptide is SEQ ID NO: 6.
78. The method of claim 75, wherein the Prion protein peptide is SEQ ID NO: 7.
79. The method of claim 75, wherein the Prion protein peptide is SEQ ID NO: 9.
80. The method of claim 75, wherein the carrier molecule is HSP70.
- 5 81. The method of claim 75, wherein the animal is a mouse.
82. The method of claim 75, wherein the screening is done using an enzyme-linked immunosorbent assay.
83. A kit for for determining if a subject is at risk for developing spongiform encephalopathy comprising:
- 10 a. at least one reagent that specifically detects Prion protein; and
- b. instructions for determining that the subject is at increased risk of developing spongiform encephalopathy.
84. A method of producing monoclonal antibodies specific to hyaluronic acid comprising:
- 15 a. conjugating the hyaluronic acid chemically to a carrier molecule;
- b. immunizing an animal with the conjugated antigen;
- c. harvesting B cells from the animal;
- d. creating a hybridoma from the harvested B cells; and
- e. screening the hybridomas for specificity to the native hyaluronic acid.
85. A method of producing monoclonal antibodies specific to matrix metalloprotease 3
- 20 comprising:
- a. conjugating the matrix metalloprotease 3 chemically to a carrier molecule;
- b. immunizing an animal with the conjugated antigen;
- c. harvesting B cells from the animal;
- d. creating a hybridoma from the harvested B cells; and
- 25 e. screening the hybridomas for specificity to the native matrix metalloprotease 3.
86. The method of claim 85, wherein the conjugating is performed chemically using glutaraldehyde.
87. The method of claim 85, wherein the carrier molecule is HSP70.
88. The method of claim 85, wherein the animal is a mouse.
- 30 89. The method of claim 85, wherein the screening is done using an enzyme-linked immunosorbent assay.

Fig. 1



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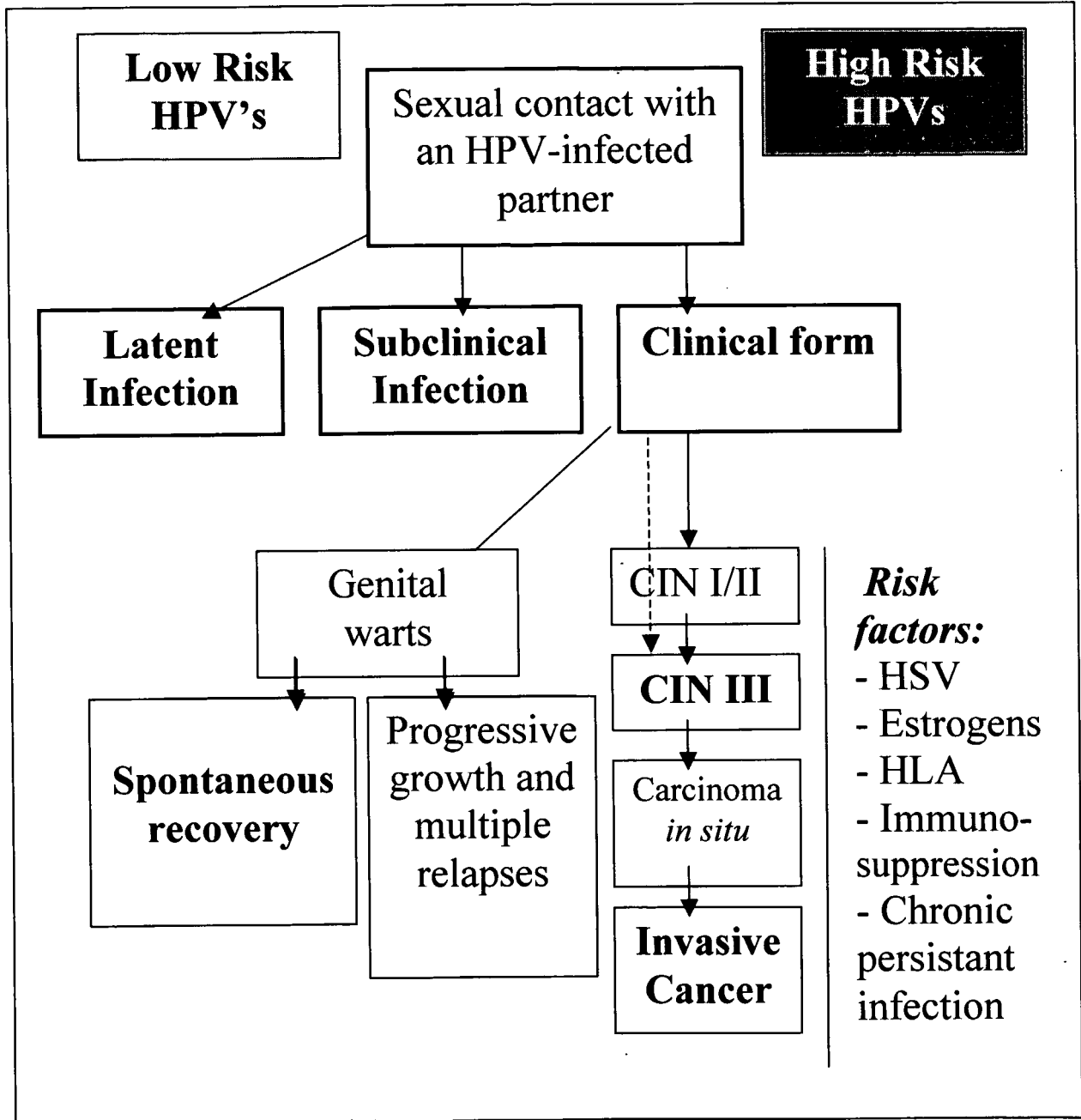


Fig. 2

18UP 5-TCT AAC GAA TTC AGT ATG CAT GGA CCT AAG G (SEQ ID NO.: 14)
 18 DOWN 5-ATT ACA GGA TCC CTG CTG GGA TGC ACA CCA (SEQ ID NO.: 15)
 16UP 5-ATT CTC GAA TTC ATC ATG CAT GGA GAT ACA C (SEQ ID NO.: 16)
 16 DOWN 5-CTT ATC GGA TCC TGG TTT CTG AGA ACA GAT G (SEQ ID NO.: 17)

Fig. 3

gaattcatcatgcatggagatacacctacattgcatgaatataatgtagatttgcaacca
 EcoRI I M H G D T P T L H E Y M L D L Q P
 gagaaactgactctactgttatgagcaatataatgacagctcagaggaggatgaa
 E T T D L Y C Y E Q L N D S S E E D E
 atagatgggtccagctggacaagcagaaccggacagagcccattacaataattgtaacctt
 I D G P A G Q A E P D R A H Y N I V T F
 tgttgcaagtgtgactctacgcttcggttgtgctacaagcacacacgtagacattcgt
 C C K C D S T L R L C V Q S T H V D I R
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 T L E D L L M G T L G I V C P I C S Q K

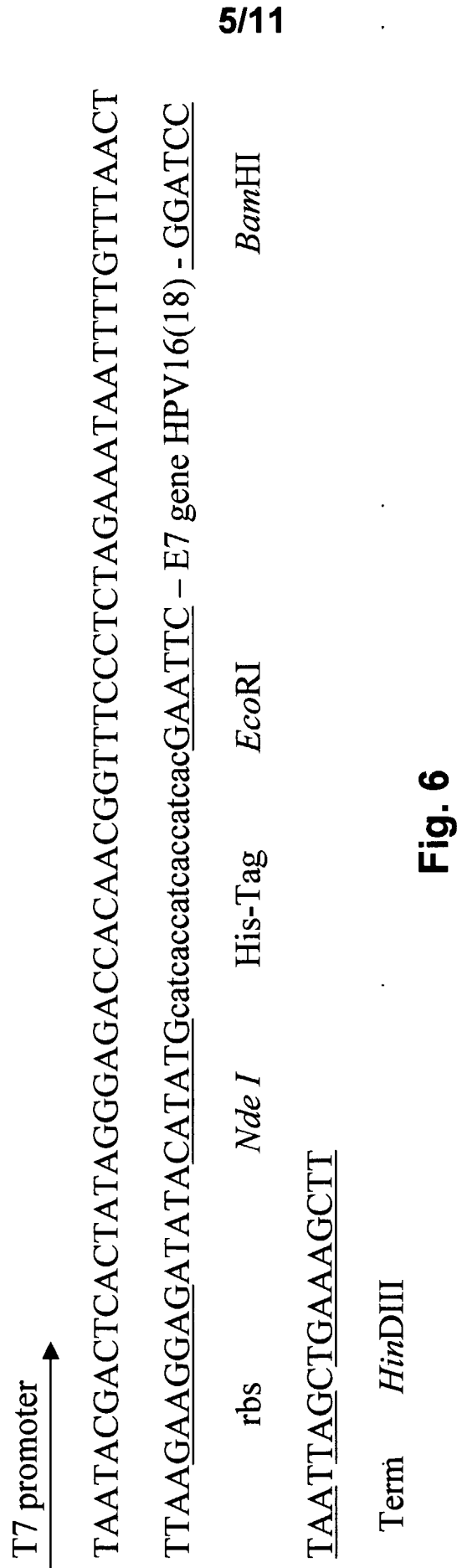
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P BamHI

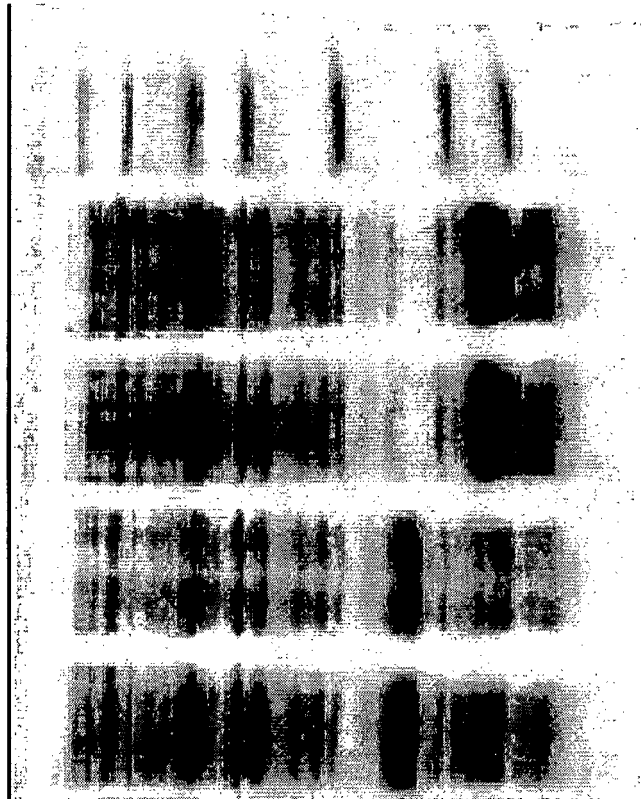
Fig. 4

gaattcagtatgcatggaccctaaggcaacattgcaagacattgtattgcatatttagagccc
 EcoRI S M H G P K A T L Q D I V L H L E P
 caaatgaaattccggttgaccttctatgtcacgagcaattaagcgaactcagaggaagaa
 Q N E I P V D L L C H E Q L S D S E E E
 aacgatgaaatagatggagttaatcaacattaccagcccagagctgaaccacaa
 N D E I D G V N H Q H L P A R R A E P Q
 cgtcacacaatgtgtatgtgttaagtgtgaagccagaattgagctagtagtagaa
 R H T M L C M C C K C E A R I E L V V E
 agctcagcagacaccttcgagcattccagcagctgtttctgaacaccctgtcctttgtg
 S S A D D L R A F Q Q L F L N T L S F V
 tgtccgtggtgtgcatcccagcag**ggatcc**
 C P W C A S Q Q BamHI

Fig. 5



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Type 16 HPV7
Type 18 HPV E7

Fig. 7

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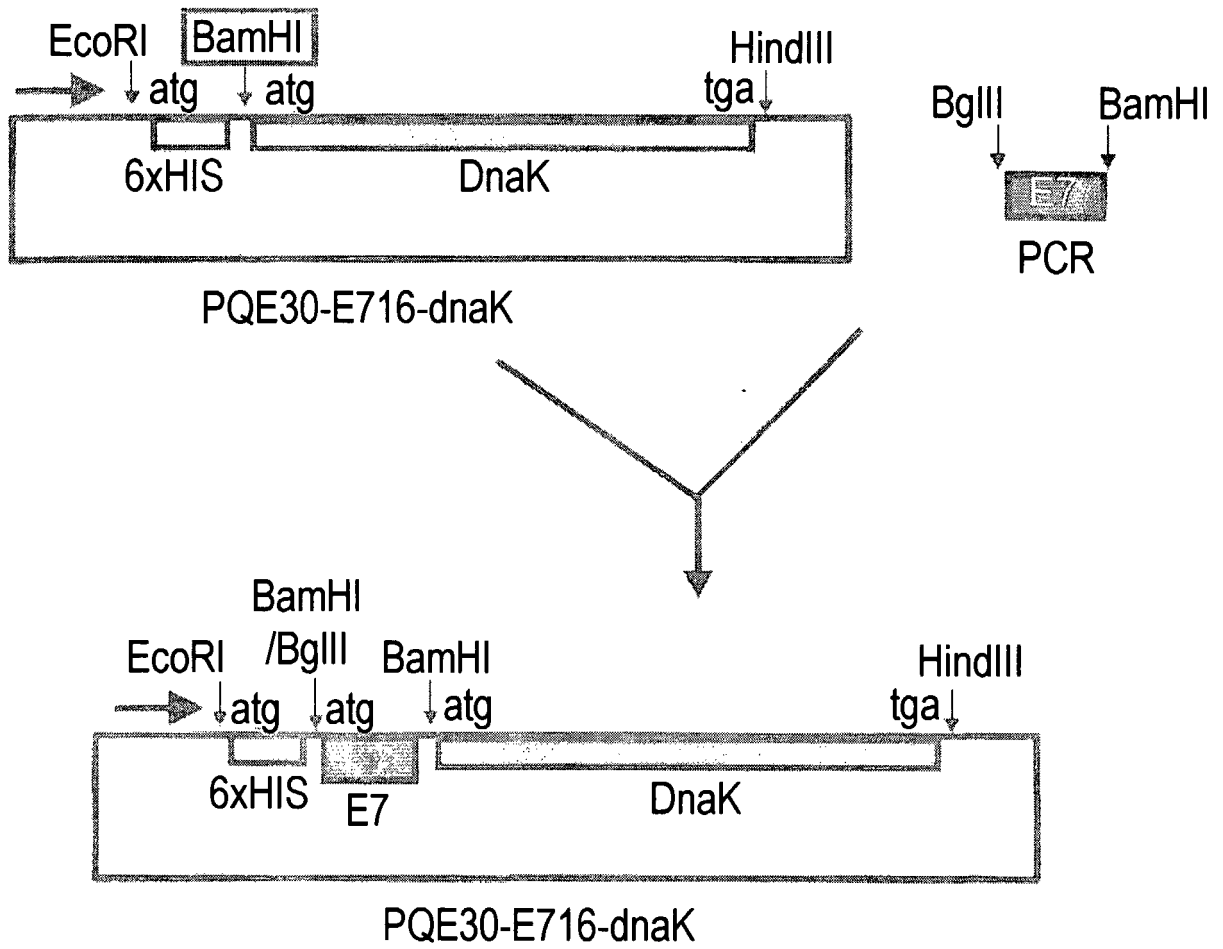


Fig. 9

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1 CTCGAGAAAT CATAAAAAAT TTATTTGCTT TGTGAGCGGA TAACAATTAT AATAGATTCA
 61 ATTGTGAGCG GATAACAATT TCACACAGAA TTCATTAAG AGGAGAAATT AACTATGAGA
 121 GGATCGCATC ACCATCACCA TCACGGATCC GCTCGTGCGG TCGGGATCGA CCTCGGGACC
 181 ACCAACTCCG TCGTCTCGGT TCTGGAAGGT GGCGACCCGG TCGTCTCGC CAACTCCGAG
 241 GGCTCCAGGA CCACCCCGTC AATTGTTCGG TTCGCCCGCA ACGGTGAGGT GCTGGTTCGGC
 301 CAGCCCGCCA AGAACCAGGC AGTGACCAAC GTCGATCGCA CCGTGCCTC GGTCAAGCGA
 361 CACATGGGCA GCGACTGGTC CATAGAGATT GACGGCAAGA AATACACCGC GCCCGAGATC
 421 AGCGCCCGCA TTCTGATGAA GCTGAAGCGC GACGCCGAGG CCTACCTCGG TGAGGACATT
 481 ACCGACGCGG TTATCACGAC GCCCGCTAC TTCAATGACG CCCAGCGTCA GGCCACCAAG
 541 GACGCCGGCC AGATCGCCGG CCTCAACGTG CTGCGGATCG TCAACGAGCC GACCGCGGCC
 601 GCGCTGGCCT ACGGCCTCGA CAAGGGCGAG AAGGAGCAGC GAATCCTGGT CTTGACTTG
 661 GGTGGTGGCA CTTTCGACGT TTCCCTGCTG GAGATCGGC AGGGTGTGGT TGAGGTCCGT
 721 GCCACTTCGG GTGACAACCA CTCGGCGGC GACGACTGG ACCAGCGGGT CGTCGATTGG
 781 CTGGTGGACA AGTTCAAGGG CACCAGCGGC ATCGATCTGA CCAAGGACAA GATGGCGATG
 841 CAGCGGCTGC GGAAGCCGC CGAGAAGGCA AAGATCGAGC TGAGTTCGAG TCAGTCCACC
 901 TCGATCAACC TGCCCTACAT CACCGTCGAC GCCGACAAGA ACCCGTTGTT CTTAGACGAG
 961 CAGCTGACCC GCGCGGAGTT CCAACGGATC ACTCAGGACC TGCTGGACCG CACTCGCAAG
 1021 CCGTTCAGT CCGTGATCGC TGACACCGGC ATTTCCGGTGT CGGAGATCGA TCACGTTGTG
 1081 CTCGTGGGTG GTTCGACCCG GATGCCCGCG GTGACCGATC TGGTCAAGGA ACTCACCGGC
 1141 GGCAAGGAAC CCAACAAGGG CGTCAACCCC GATGAGGTTG TCGCGGTGGG AGCCGCTCTG
 1201 CAGGCCGGCG TCCTCAAGGG CGAGGTGAAA GACGTTCTGC TGCTTGATGT TACCCGCTG
 1261 AGCCTGGGTA TCGAGACCAA GGGCGGGGTG ATGACCAGGC TCATCGAGCG CAACACCACG
 1321 ATCCCCACCA AGCGGTCGGA GACTTTCACC ACCGCCGACG ACAACCAACC GTCGGTGCAG
 1381 ATCCAGGTCT ATCAGGGGGA GCGTGAGATC GCCGCGCACA ACAAGTTGCT CGGGTCCTTC
 1441 GAGCTGACCG GCATCCCGCC GGCGCCGCGG GGGATTCGC AGATCGAGGT CACTTTCGAC
 1501 ATCGACGCCA ACGGCATTGT GCACGTCACC GCCAAGGACA AGGGCACCAG CAAGGAGAAC
 1561 ACGATCCGAA TCCAGGAAGG CTCGGGCCTG TCCAAGGAAG ACATTGACCG CATGATCAAG
 1621 GACGCCGAAG CGCACGCCGA GGAGGATCGC AAGCGTCGCG AGGAGGCCGA TGTTCGTAAT
 1681 CAAGCCGAGA CATTGGTCTA CCAGACGGAG AAGTTCGTCA AAGAACAGCG TGAGGCCGAG
 1741 GGTGGTTCGA AGGTACCTGA AGACACGCTG AACAAGGTTG ATGCCGCGGT GGCAGGAGCG
 1801 AAGGCGGCAC TTGGCGGATC GGATATTTTCG GCCATCAAGT CGGCGATGGA GAAGCTGGGC
 1861 CAGGAGTCGC AGGCTCTGGG GCAAGCGATC TACGAAGCAG CTCAGGCTGC GTCACAGGCC
 1921 ACTGGCGCTG CCCACCCCGG CGGCGAGCCG GGCGGTGCC ACCCCGCTC GGCTGATGAC
 1981 GTTGTGGACG CGGAGGTGGT CGACGACGGC CGGGAGGCCA AGTGACGGAC GGGTCGACCT
 2041 GCAGCCAAGC TTAATTAGCT GAGCTTGAC TCCTGTTGAT AGATCCAGTA ATGACCTCAG
 2101 AACTCCATCT GGATTTGTTT AGAACGCTCG GTTGCCGCGG GCGTTTTTTT ATTGGTGAGA
 2161 ATCCAAGCTA GCTTGCGGAG ATTTTCAGGA GCTAAGGAAG CTAAAATGGA GAAAAAATC
 2221 ACTGGATATA CCACCGTTGA TATATCCCAA TGGCATCGTA AAGAACATTT TGAGGCATTT
 2281 CAGTCAGTTG CTCAATGTAC CTATAACCAG ACCGTTTCAGC TGGATATTAC GGCTTTTTTA
 2341 AAGACCGTAA AGAAAAATAA GCACAAGTTT TATCCGGCCT TTATTCACAT TCTTGCCCGC
 2401 CTGATGAATG CTCATCCGGA ATTTTCGTATG GCAATGAAAG ACGGTGAGCT GGTGATATGG
 2461 GATAGTGTTT ACCCTTGTTA CACCGTTTTT CATGAGCAA CTGAAACGTT TTCATCGCTC
 2521 TGGAGTGAAT ACCACGACGA TTTCCGGCAG TTTCTACACA TATATTCGCA AGATGTGGCG
 2581 TGTTACGGTG AAAACCTGGC CTATTTCCCT AAAGGGTTTA TTGAGAATAT GTTTTTTCGTC
 2641 TCAGCCAATC CCTGGGTGAG TTTACCAGT TTTGATTTAA ACGTGGCCAA TATGGACAAC
 2701 TTCTTCGCC CCCTTTTCAC CATGGGCAA TATTATACGC AAGGCGACAA GGTGCTGATG
 2761 CCGCTGGCGA TTCAGTTCA TCATGCCGTT TGTGATGGCT TCCATGTCGG CAGAAATGCTT
 2821 AATGAATTAC AACAGTACTG CGATGAGTGG CAGGGCGGGG CGTAATTTTT TTAAGGCAGT
 2881 TATTGGTGCC CTTAACGCC TGGGGTAATG ACTCTCTAGC TTGAGGCATC AAATAAACG

Fig. 10

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 3181 ACCCAGTCAC GTAGCGATAG CGGAGTGTAT ACTGGCTTAA CTATGCGGCA TCAGAGCAGA
 3241 TTGTACTGAG AGTGCACCAT ATGCGGTGTG AAATACCGCA CAGATGCGTA AGGAGAAAAT
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 3481 CCGCGTTGCT GCGGTTTTTC CATAGGCTCC GCCCCCTGA CGAGCATCAC AAAAAATCGAC
 3541 GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG
 3601 GAAGCTCCCT CGTGCGCTCT CCGTGTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT
 3661 TTCTCCCTTC GGGAAGCGTG GCGTTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG
 3721 TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCCTCAG CCCGACCGCT
 3781 GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC
 3841 TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT
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 4201 AAAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC
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 4981 CGATGTAACC CACTCGTGCA CCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT
 5041 CTGGGTGAGC AAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG GCGACACGGA
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 5161 GTCTCATGAG CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA GGGGTTCCGC
 5221 GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC ATGACATTAA
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Fig. 10

11/11

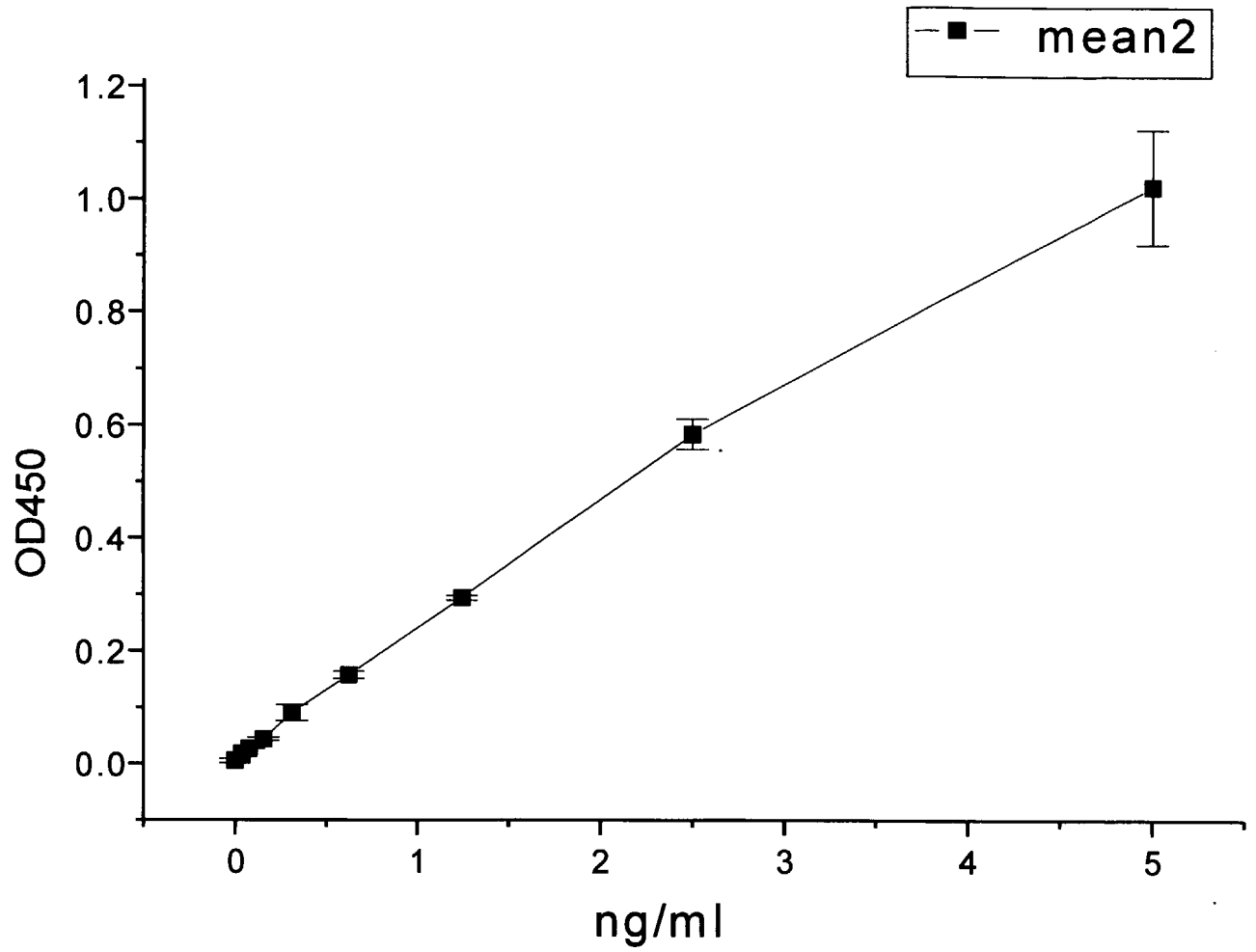


Fig. 11

Immunize.ST25.txt

SEQUENCE LISTING

<110> Kiselev, Vsevolod I
Petr, Sveshnikov G

<120> METHODS, KITS, AND COMPOSITIONS FOR THE DEVELOPMENT AND USE OF
MONOCLONAL ANTIBODIES SPECIFIC TO ANTIGENS TRADITIONALLY OF LOW
IMMUNOGENICITY

<130> Immunize

<150> RU 2003128660

<151> 2003-09-25

<160> 22

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Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Cys Tyr Glu Gln Leu Asn
15 20 25 30

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Asp Ser Ser Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala
35 40 45

gaa ccg gac aga gcc cat tac aat att gta acc ttt tgt tgc aag tgt 192
Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys
50 55 60

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Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg
65 70 75

act ttg gaa gac ctg tta atg ggc aca cta gga att gtg tgc ccc atc 288
Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile
80 85 90

tgt tct cag aaa cca ggatcc 309
Cys Ser Gln Lys Pro
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Immunize.ST25.txt

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 35 40 45

Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser
 50 55 60

Thr Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu
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Gln Lys Pro

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cat tta gag ccc caa aat gaa att ccg gtt gac ctt cta tgt cac gag 96
 His Leu Glu Pro Gln Asn Glu Ile Pro Val Asp Leu Leu Cys His Glu
 15 20 25 30

caa tta agc gac tca gag gaa gaa aac gat gaa ata gat gga gtt aat 144
 Gln Leu Ser Asp Ser Glu Glu Glu Asn Asp Glu Ile Asp Gly Val Asn
 35 40 45

Immunize.ST25.txt

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 Leu Cys Met Cys Cys Lys Cys Glu Ala Arg Ile Glu Leu Val Val Glu
 65 70 75

agc tca gca gac gac ctt cga gca ttc cag cag ctg ttt ctg aac acc 288
 Ser Ser Ala Asp Asp Leu Arg Ala Phe Gln Gln Leu Phe Leu Asn Thr
 80 85 90

ctg tcc ttt gtg tgt ccg tgg tgt gca tcc cag cag ggatcc 330
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- <210> 4
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- <212> PRT
- <213> Human papillomavirus type 18

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Glu Pro Gln Asn Glu Ile Pro Val Asp Leu Leu Cys His Glu Gln Leu
 20 25 30

Ser Asp Ser Glu Glu Glu Asn Asp Glu Ile Asp Gly Val Asn His Gln
 35 40 45

His Leu Pro Ala Arg Arg Ala Glu Pro Gln Arg His Thr Met Leu Cys
 50 55 60

Met Cys Cys Lys Cys Glu Ala Arg Ile Glu Leu Val Val Glu Ser Ser
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Phe Val Cys Pro Trp Cys Ala Ser Gln Gln
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- <213> Artificial Sequence

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 <223> Nucleotide sequence of recombinant vector pQE30-dnaK

Immunize.ST25.txt

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Immunize.ST25.txt

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Immunize.ST25.txt

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Immunize.ST25.txt

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<210> 13
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专利名称(译)	用于开发和使用对低免疫原性抗原特异的单克隆抗体的方法，试剂盒和组合物		
公开(公告)号	EP1673393A2	公开(公告)日	2006-06-28
申请号	EP2004775280	申请日	2004-09-24
[标]申请(专利权)人(译)	基谢廖夫弗谢沃洛德·伊万诺维奇·SVESHNIKOV PETR格奥尔基耶		
申请(专利权)人(译)	基谢廖夫，弗谢沃洛德·伊万诺维奇·SVESHNIKOV，PETR格奥尔基耶		
当前申请(专利权)人(译)	基谢廖夫，弗谢沃洛德·伊万诺维奇·SVESHNIKOV，PETR格奥尔基耶		
[标]发明人	KISELEV VSEVOLOD IVANOVICH SVESHNIKOV PETR GEORGIYEVICH		
发明人	KISELEV, VSEVOLOD IVANOVICH SVESHNIKOV, PETR GEORGIYEVICH		
IPC分类号	C07K16/00 C07K16/08 C07K16/44 C07K16/40 A61K39/385 G01N33/574 G01N33/53 A61K39/00 A61K39/12 C07K16/18 C07K16/28 G01N33/577		
CPC分类号	A61P31/12 A61P35/00 A61P37/00 C07K16/084 C07K16/18 C07K16/2872 C07K16/40 C07K16/44 G01N33/5748		
优先权	2003128660 2003-09-25 RU		
其他公开文献	EP1673393B1		
外部链接	Espacenet		

摘要(译)

本发明涉及对传统上具有低免疫原性的抗原特异的单克隆抗体的开发和使用。本发明提供了通过将感兴趣的抗原与载体分子化学缀合来产生单克隆抗体的方法，所述载体分子使免疫系统能够对缀合的抗原进行免疫应答。本发明还提供了缀合抗原的特定组合物以及对那些缀合抗原特异的单克隆抗体。本发明还提供了用于使用单克隆抗体检测疾病状况的试剂盒。