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(54) **METHODS FOR THE IMMUNOLOGICAL IDENTIFICATION OF CEREBROSPINAL FLUID**
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Description

FIELD OF THE DISCLOSURE

5 **[0001]** The present disclosure relates to detection of the presence or absence of cerebrospinal fluid (CSF) in a sample by the detection of one or more proteins that are enriched in CSF compared to their levels in other bodily fluids. Described herein are devices and methods for the detection of the presence or absence of cerebrospinal fluid in samples of mixed bodily fluids from a wide variety of human populations crossing ethnicity, age, gender, health status and genetic variability.

10 BACKGROUND

[0002] Cerebrospinal fluid (CSF), or liquor cerebrospinalis, is found in the subarachnoid space as well as in the ventricles surrounding and penetrating the central nervous system (CNS). CSF bathes the brain and spinal cord and provides hydrative, nutritive, metabolic waste removal, and hydrostatic impact buffer to neurons and glia. CSF is produced from arterial blood by the choroid plexuses of the lateral and fourth ventricles by a combined process of diffusion, pinocytosis and active transfer. The fluid also contains constituents produced by neurons and glia. After diffusion through the ventricular system into the subarachnoid space, most of the CSF is reabsorbed by the arachnoid granulations to reenter the blood stream via the dural venous plexus. Approximately 500 ml of liquor is generated every day; with a total volume of 140-150 ml for an adult, the whole CSF is renewed every 6-8 hours. The CSF is bounded by the dura throughout the CNS. More fluid is produced in the rostral CNS and more ultimately drains in the caudal spinal cord to produce a net rostral to caudal fluid flow. CSF is an isotonic mixture mostly of salts, glucose, protein and water. CSF from the lumbar region contains 15 to 45 mg/dl protein (0.3-1% of serum protein concentration) and 50-80 mg/dl glucose (60% of blood glucose). Protein concentration in cisternal and ventricular CSF is lower.

[0003] The protein landscape of the CSF can be divided into two groups: Blood derived proteins, which make up the main fraction in the CSF of healthy individuals, and brain derived proteins. Approximately 20% of the proteins in the CSF originate from the brain parenchyma, but only a subset of those are actually brain specific.

[0004] Despite the fact that the majority of liquor proteins are also found in the serum, there are multiple sources for proteins unique to the CSF:

Proteins that are released from neurons and glial cells, e.g. tau protein, S-100, and neuron-specific enolase (NSE).

30 **[0005]** Proteins released from leptomeninges, e.g. β -trace protein and cystatin C.

[0006] Proteins differentially modified by glycosylation or phosphorylation during synthesis in the choroid plexus, e.g. transthyretin (TTR), angiotensin II, and Insulin-like growth factor II.

[0007] There is substantial overlap in the protein profile between CSF and plasma, a considerable number of proteins are unique to the CSF or are uniquely modified by phosphorylation or glycosylation in the CNS.

35 **[0008]** WO 01/63295 discloses a protein isoform related to Dickkopf-3 (DKK-3), DPI-6, and its use in the diagnosis, prophylaxis and treatment of neuropsychiatric and neurological conditions such as Bipolar Affective Disorder, Schizophrenia and Vascular Dementia.

[0009] WO 2007/047796 provides methods for identifying tissue-derived glycoproteins and glycosites in plasma, panels of detection reagents for detecting same, as well methods for detecting disease using such panels. The invention further provides a database of tissue-derived glycoproteins and glycosites detectable in plasma. DKK-3 is listed as one example (SEQ ID NO. 9863 of 14918).

[0010] Lateral Flow Tests, or also known as Lateral Flow Immunochromatographic Assays or Strip Tests, are designed to rapidly detect the presence or absence of a given analyte in a heterogenous matrix. A variety of Lateral Flow Tests are currently on the market for home testing, point of care testing, or laboratory use, for instance pregnancy tests (e.g., FirstResponse®, ClearBlue®), HIV tests (e.g., OraQuick ADVANCE®, Clearview® Complete), or Chlamydia tests (e.g., Clearview® Chlamydia, inSTIcheck™ Chlamydia).

[0011] What is needed is a test suitable for detection of CSF that is comparable to HIV tests like OraQuick ADVANCE® or Clearview® Complete: It is a point of care test; the test is only qualitative; the operator needs minimal training to use the test; the test has an internal control on the strip to verify accurate sampling.

50 SUMMARY

[0012] In one aspect the invention provides the use of a device in an in vitro method for detecting the presence or absence of cerebrospinal fluid (CSF) in a sample, said method comprising contacting the sample with a binding partner specific for a CSF-enriched protein, and detecting binding partner-CSF-enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of said CSF-enriched protein in the sample; wherein the CSF-enriched protein is cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number

gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched protein, said device comprising

a sample application region,

a sample labeling region comprising a first antibody to said CSF-enriched protein, wherein the first antibody is conjugated to a mobile particle;

a sample detection region comprising a second antibody to said CSF-enriched protein, wherein the second antibody is fixed to the sample detection region,

wherein the formation of a detectable band in the second region following application of the sample to the sample application region indicates the presence of said CSF-enriched protein in the sample.

In one aspect the invention provides an *in vitro* method for detecting the presence or absence of CSF in a sample, said method comprising

contacting the sample with a binding partner specific for a CSF-enriched protein, and

detecting binding partner-CSF-enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of said CSF-enriched protein in the sample;

wherein the CSF-enriched protein is cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched protein.

[0013] Also described herein is a device for detection of the presence or absence of cerebrospinal fluid in a sample comprises

a sample application region,

a sample labeling region comprising a first antibody to a CSF-enriched protein, wherein the first antibody is conjugated to a mobile particle;

a sample detection region comprising a second antibody to the CSF-enriched protein, wherein the second antibody is fixed to the sample detection region,

wherein the presence of a detectable band in the second region indicates the presence of cerebrospinal fluid in the sample.

[0014] Also described herein is a method for detecting the presence or absence of CSF in a sample, comprises

contacting the sample with a binding partner specific for a CSF-enriched protein, and

detecting binding partner-CSF enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of CSF in the sample.

[0015] In the foregoing embodiments, the CSF antigen is Isoform 1 of Neural cell adhesion molecule-like (SEQ ID NO: 1; Accession Number gi:62088238) protein; Chain A, Human Mesotrypsin Complexed With Bovine Pancreatic Trypsin Inhibitor(Bpti) (SEQ ID NO:2; Accession number gi:162330095); CNTN2 Contactin-2 precursor (SEQ ID NO: 3; Accession Number gi|4827022); CNTN1 Isoform 2 of Contactin-1 (SEQ ID NO: 4; Accession Number gi:28373119);

cDNA highly similar to SPARC-like protein 1 (unnamed protein product) (SEQ ID NO: 5; Accession Number: gi|194388050); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (SEQ ID NO: 6; Accession Number: gi|68534652 and SEQ ID NO: 7; gi|109731501); NCAM2 Neural cell adhesion molecule 2, isoform CRA_a (SEQ ID NO: 8; Accession Number gi|119630409); SERPINA3 serpin peptidase inhibitor, clade A, member 3 precursor /Isoform 1 of Alpha-1-antichymotrypsin/growth-inhibiting protein 25 [Homo sapiens] or slightly longer fragment of alpha-1-antichymotrypsin precursor (SEQ ID NO: 9; Accession Number gi|46981961);

AGT Angiotensinogen (SEQ ID NO: 10; Accession Number gi|553181); Angiotensinogen precursor (Serpina8) (SEQ ID NO: 11; Accession Number gi|4557287); unnamed protein product also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 12; Accession Number gi|187608363); cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); SERPINF1 serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor, Pef), member 1 isoform 4 factor (SEQ ID NO: 14; Accession Number gi|15988024); human protein similar to GC Vitamin D-binding protein PREDICTED: vitamin D-binding protein [Pan troglodytes] (SEQ ID NO: 15; Accession Number 181482); CD14 Human monocyte antigen CD14 (CD14) (SEQ ID NO: 16; Accession Number gi|117646212); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (SEQ ID NO: 17; Accession Number gi|90080503; SEQ ID NO: 18; gi|187608363 (human); Neural cell adhesion molecule variant (SEQ ID NO: 19; Accession Number gi:62088238); unnamed protein similar to CLU cDNA FLJ57622, highly similar to Clusterin (SEQ ID NO: 20; Accession number gi|189054091); protein highly similar to Clusterin (SEQ ID NO: 21; Accession number gi|193787502); LMAN2 Vesicular integral-membrane protein VIP36 (SEQ ID NO: 22; Accession number gi|157834800); clusterin isoform 1 [Homo sapiens] (SEQ ID NO: 23; Acces

sion number NM_001831.2); superoxide dismutase 3, extracellular precursor (SEQ ID NO: 24; Accession number gi|118582275); fibrin alpha C term fragment (SEQ ID NO: 25; Accession number gi|223057); Chain A, Human Kallikrein 6 (Hk6) Active Form or KLK6 Isoform 1 of Kallikrein-6 (SEQ ID NO: 26; Accession number gi|21465970); APCS Serum amyloid P-component /Chain A or Pentameric Human Serum Amyloid P Component (SEQ ID NO: 27; Accession number gi|576259); FAM3C Protein FAM3C / family with sequence similarity 3, member C precursor [Homo sapiens] note="predicted osteoblast protein; interleukin-like EMT inducer (SEQ ID NO: 28; Accession number gi|55629272); protein similar

to unnamed protein product [Macaca fascicularis] also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 29; Accession number gi|187608363); a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF antigens; or a combination of two or more of the foregoing CSF antigens.

[0016] Also described herein is a method for the detection of a reactant in a body fluid, tissue or microorganism comprises contacting the body fluid, tissue or microorganism with two or more antibodies, wherein each antibody specifically reacts with an antigen in the reactant, wherein reaction with each individual antibody does not indicate a positive test for the reactant, and wherein reaction with the two or more antibodies indicates a positive test for the reactant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017]

Figure 1 Lateral Flow assay. Analyte is added to the left end of the strip either by a dropper or by direct dipping. The liquid (around 75 μ l) is wicked across the strip to the right. The conjugate pad contains soluble IgG attached to a visible particle (i.e., gold or latex microspheres). If the analyte solution contains the analyte, the antibodies conjugate and the complex migrates across the strip. The mixture first encounters the test strip, which contains immobilized antibody against the analyte. The analyte, soluble primary and visible tag, then bind to the test line. If no analyte is present the soluble fraction passes over the test line. Whether the analyte is present or not, excess soluble IgG bound to indicator binds to the immobilized anti-globin IgG bound to the control strip.

Figure 2 shows advantages of a multi antigen approach to CSF detection. The upper figure represents single antigen assay results for various test conditions and the bottom figure shows results of the multi antigen assay. The bars along the X axis represent different assay conditions and the Y axis represents the degree of immunoreactivity seen by the assay. The upper shaded zone indicates a positive colorimetric response on the test line of the lateral flow assay. Assays with immunoreactivity that enters the shaded zone will produce a positive test result. Bar 1: CSF Bars in the upper graph illustrate immunoreactivity of the single antigen being sufficient to produce a positive test result. Alternatively in the multiple antigen graph (lower) a combination of antigens, each producing a partial signal accumulates to produce positive assay result. Bar 2: CSF contaminated with blood produces a similar positive response with a smaller but additive blood immunoreactivity (upper bar with thick border). Bar 3: Unusual CSF/blood sample in which antigen 1 is poorly immunoreactive. In the single antigen assay, the assay produces a false negative, while the multi antigen assay is still above assay threshold as a result of the other five antigen immunoreactivities being intact. Bar 4: CSF/blood with no antigen 1 immunoreactivity. Same results as in Bar 3. Bar 5: No CSF but blood borne cross-reactive antigen. In this case the single antigen assay produces a false positive, but as the immunoreactivity of the single antigen is not sufficient to produce a positive signal in the multi antigen assay the assay reports the correct negative result. Bar 6: No CSF but blood level of antigen 1 pathologically high. Single antigen assay produces false positive reacting to heightened blood levels. Multi antigen assay reacts to pathogenic antigen 1 levels in blood but does not reach threshold for false positive. This assay is shown with 5 antigen/antibody/antibody2 mixes, however other embodiments could contain between 2 and as many as 10 antigen/antibody/antibody2 mixes.

Figure 3: Two dimensional gel electrophoresis of CSF and blood proteins. An example of a single experiment in which 100 μ g of Cy- tagged CSF protein (A) and 100 μ g of Cy3-tagged blood proteins (B) are separated in two dimensions. A and B are grayscale images of the same gel using different excitation and emission settings. The pH range is 4-8. C) is the RGB merge of the two channels with yellow spots indicating significant overlap. D) is an automated extraction of spots with >5x enrichment in either the CSF or blood. All samples were 2x depleted of major serum/CSF proteins (see Methods).

Figure 4: Liquid chromatography-mass spectroscopy analysis of some of the CSF-enriched spots seen on the gel in Figure 3.

Figure 5: CSF-enriched proteins FLJ55 and dickkopf homolog 3 precursor (DKK3). A) Immunoblot of FLJ55. Affinity purified polyclonal rabbit anti-human antibody produced against a recombinant fragment of FLJ55 produces immunoreactivity at the correct molecular weight in the CSF sample but not in the serum sample. B) Affinity purified polyclonal rabbit anti-human antibody produced against a recombinant fragment of DKK3 also produces immunoreactivity at the correct molecular weight in the CSF sample but not in the serum sample. In both cases excessive serum protein was loaded at levels higher than that of the sera. C) Four separate samples of CSF indicating immunoreactivity for DKK3 with a different affinity purified antibody (left). Five blood samples fail to produce immunoreactivity. Lane 5 blood is high non specific background.

Figure 6: Phosphorylated forms of angiotensinogen that are highly enriched in the CSF. An RGB merge of the Cy3 blood (green) and Cy5 CSF (red). We have identified several novel and non-overlapping phosphorylated versions (right four red spots) that are not present in the blood. At least three other combinations (left three spots) are present in both CSF and blood.

Figure 7: CSF specific post translational modifications. Change in the CSF 2D gel protein distribution pattern before (top panel) and after (middle panel) removal of all secondary modifications of the extracted proteins. Red spots in lower panel indicate a reduction in a particular protein signal following removal of the post-translational modification.
 Figure 8: Experimental flow chart for the production of CSF detection test strips.

Figure 9: CSF proteins that are phosphorylated. A single DIGE gel in which two samples of serum protein depleted CSF was run. A) the Cy3 labeled proteins from the CSF sample which was incubated in alkaline phosphatase for one hour. B) Equivalent sample of serum protein depleted CSF not treated with alkaline phosphatase. C) Computer generated difference (blue boundaries) between spot volume of the two gels (A vs B). All blue spots represent phosphorylated CSF proteins.

DETAILED DESCRIPTION

[0018] Described herein are proteins that are enriched in CSF compared to other bodily fluids and methods for the detection of the presence or absence of cerebrospinal fluid (CSF) in a sample by the detection of these proteins. Also described herein are devices and methods for the detection of the presence or absence of CSF in samples of mixed bodily fluids from a wide variety of human populations crossing ethnicity, age, gender, health status and genetic variability. The CSF-enriched proteins are detected with a specific protein binding partner such as an antibody, a ligand, a receptor, and the like. Binding partners can be natural or synthetic binding partners.

[0019] Binding can be detected either directly, or indirectly, such as with a fluorescent label attached to the binding partner. While several embodiments are included that use antibodies as binding partners, it should be understood that other binding partners can be used in place of antibodies.

[0020] In certain embodiments, the level of the CSF-enriched protein is quantitated. Such quantitation is particularly useful in the identification of brain injury. Quantitation can be performed by using a binding partner with a detectable label. "Detectable moiety" or a "label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Useful labels include ³²P, ³⁵S, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin-streptavidin, dioxigenin, haptens and proteins for which antisera or monoclonal antibodies are available. The detectable moiety often generates a measurable signal, such as a radioactive, chromogenic, or fluorescent signal that can be used to quantitate the amount of bound detectable moiety in a sample. The detectable moiety can be incorporated in or attached to a binding partner either covalently, or through ionic, van der Waals or hydrogen bonds. The detectable moiety may be directly or indirectly detectable. Indirect detection can involve the binding of a second directly or indirectly detectable moiety to the detectable moiety.

[0021] In some embodiments, CSF detection is performed using a lateral flow assay, employing for example, antibodies specific for the CSF protein of interest. A lateral flow assay can be a single antigen assay or a multiple antigen assay. A multiple antigen test uses all of the antigens together to provide a single easy to read answer (i.e., a single band on a strip assay). A multiple antigen test may qualify or quantify each of several antigens individually to give a more complex profile of the antigens that are present. Such a profile may be useful to determine the severity of a head injury, that is, the head injury is less severe when certain CSF-specific proteins are present and more severe when other CSF-specific proteins are present or levels of each protein provides a degree of injury.

Single antigen assay:

[0022] While lateral flow technology has been successfully used in many clinical assays, the unique and innovative approach described herein extends the technology to i.) bind single or multiple CSF-enriched proteins, thereby increasing sensitivity and specificity of the test, and/or ii.) detect a CSF-specific post-translational modification (e.g., phosphorylation).

[0023] As used herein, a CSF-enriched protein or CSF antigen or polypeptide is an antigen or polypeptide that is specific for CSF or substantially enriched in CSF compared to other bodily fluids. Table 1 identifies several proteins known to be concentrated in the CSF. These are not proteins identified in the current application, although they can, in some embodiments, be combined in an assay with one or more proteins identified herein in a multi-antigen assay.

Table 1

Protein	MW (kDa)	CSF concentration	CSF/serum ratio
β-trace protein	25	16.6 mg/l	34:1
Cystatin C	13.3	3.1 mg/l	5:1
Tau-protein	55-74	0.2 μg/l	10:1

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(continued)

Protein	MW (kDa)	CSF concentration	CSF/serum ratio
S-100 B	21	1.5 µg/l	18:1
NSE	78	8 mg/l	1:1
Transthyretin	55	17 mg/l	1:18
Albumin	67	245 mg/l	1:205
IgG	150	25 mg/l	1:440

[0024] Described herein are proteins that are present in sufficient quantities and enriched significantly in CSF compared to their levels in other bodily fluids, to act as a marker of CSF. The proteins found in pooled samples of CSF were compared to the proteins in blood, nasal fluid, saliva, sweat, tears and ear effluents (referred to as 'other bodily fluids'). CSF from a range of ages (1-70 years) and from both males and females was examined. Prior to comparative 2D gel electrophoresis, all fluids were treated to remove dominant serum proteins that are present in most bodily fluids (i.e., albumin, IgG, etc.). The remaining proteins from CSF and another bodily fluid were differentially tagged with Cy3 and Cy5 and run on two-dimensional PAGE. Using this approach, a novel set of proteins which are highly concentrated in the CSF over other bodily fluids were identified. CSF-enriched secondary modified proteins (i.e., phosphorylated) have also been identified. Dephosphorylation of CSF extracts confirmed that the CSF unique spots represent differential migration in the isoelectric dimension based on phosphorylation.

[0025] In one embodiment, cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof and optionally the other proteins that are enriched in CSF described herein are used to detect CSF in an assay, such as a lateral flow assay. A lateral flow system consists of overlapping membranes containing the dried components needed for the test performance (Figure 1). These membranes are assembled to small strips which can be placed into a plastic housing for better handling. The patient's material is loaded to the *Sample Pad*. In the case of whole blood/capillary blood samples a separation of blood cells and plasma takes place. The liquid fraction of the patient's sample diffuses through the *Conjugate Pad* containing labeled antibodies, which are specifically directed against the analyte of interest. The antibodies (conjugate) are re-dissolved and the analyte is specifically bound by the gold (or latex) conjugate. The analyte-gold-conjugate complex further diffuses through the *Analytical Membrane*. On this membrane two lines are arranged one after the other: (i) the **Test Line** containing a second set analyte-specific antibodies responsible for immobilizing the analyte-gold conjugate complexes and (ii) the **Control Line** fixing non-bound gold antibodies indicating that the conjugate has overflowed the Test Line. If the analyte of interest is available above the detection limit the Test Line and the Control Line are clearly visible; if the analyte is below the detection limit only the Control Line appears during test time. The last component of the rapid test is the *Wicking (or Sink) Pad* which simply collects the fluid running through the test system and preventing backflow of the fluid through the test system.

[0026] Lateral Flow Immunochromatographic Assays are designed either as sandwich assays or as competitive assays. Sandwich assays makes use of two different antibodies raised against the same analyte, one to color the analyte and one to concentrate the analyte at the test line. The test line will show as a colored band in positive samples. Competitive assays provide already colored analyte on the test strip and a set of antibodies against the analyte at the test line. The sample flows with the provided colored analyte towards the test line and competes for antibody binding. The test line will show as a colored band in negative samples.

CSF Assay Design Specifications:

[0027] The assay described herein can be used to accurately identify traces of CSF when it is mixed with a variety of non-CSF bodily fluids. These 'other fluids' are, for example, nasal and ear effluents, saliva, tears, sweat, urine and blood. The assay is intended to minimize false positive or false negative results regardless of the physiologic, metabolic or pathologic state, gender, age or ethnicity of the subject.

[0028] In one embodiment, the limit of detection is >5% CSF in a pure fluid or mixture of any of the above fluids. It may be possible to achieve a higher sensitivity but it will be essential to maintain the specificity in addition to the increased sensitivity. Thus, in some embodiments, a limit of detection of >1% CSF is achieved.

Multi antigen CSF 'tissue' assay:

[0029] In one embodiment, the assay is one that will allow the detection of the presence of CSF via simultaneous

detection of multiple CSF-enriched proteins including cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof. That is, the test includes two or more markers for CSF to provide improved reliability of CSF detection. Rather than testing for a single 'biomarker', the multiple marker assay will be robust and provide the correct answer under a variety of potential and unknown circumstances with high selectivity and sensitivity. For example, a single antigen assay may produce a false positive if the antibody recognizes an antigen in a fluid other than CSF (i.e. blood). If the assay tests for an antigen which is 'enriched' in CSF but not 'exclusive' to CSF, an aberrantly high blood level could produce a false positive. This may be problematic because it is not feasible to test the strip under all possible physiologic, pathologic, ethnic, sex, dietary, age-related, etc. conditions to look for false results. Further, the level of particular CSF antigen may be reduced below detection level, or a particular CSF antigen may have a rare genotypic difference, thus reducing reactivity in certain human populations thereby producing a false negative. These are all potential difficulties that arise from basing a test on a single CSF-enriched antigen (see Figure 2). The novel 'Multi antigen' assay for detecting CSF in mixed body fluid samples should provide substantial improvement over single-antigen tests. In certain embodiments, the multi-antigen test includes at least one antibody specific for each of 2, 3, 4, 5, 6, 7, 8, 9 or 10 antigens that are enriched in CSF compared to their levels in other bodily fluids including cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof. In other embodiments, at least two antibodies specific for each antigen including cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof are employed.

[0030] As described herein, a large number of CSF-enriched protein spots have been extracted and analyzed by LC-MS. The rationale for this approach is illustrated in Figure 2. Several CSF-enriched antigens have been identified and at least two different antibodies have been produced to each antigen. Mixtures of each of the two sets of IgG are added to the mobile and immobilized portions of the test strip (see Figure 2), respectively. The multi antigen assay works by applying a concentration of antibodies for a particular antigen that are below the threshold for detection when all antibody molecules are bound. A mixture of several antibodies each a subthreshold levels are utilized in the assay. When CSF is added, all antibodies bind and accumulate producing a positive signal. The optimal embodiment would use at least 5-6 different antigens with a detection threshold of 4 so loss of a single antigen will not cause a false negative. In one embodiment, the device or test comprises 4 to 10 different antibodies that each specifically binds a different CSF antigens including cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof, wherein a positive test does not require binding to all antibodies. Accumulation of IgG/antigen on the test strip is linear and subthreshold levels for individual detection of each antibody are used then only the addition of other positive antibodies will produce a positive reaction. A positive response requiring accumulation of at least 4 IgG/antigens the assay will be more robust in the face of fluctuations in the levels of any one antigen. The assay will also be more robust in the face of aberrant increases in single antigen immunoreactivity in contaminating bodily fluids. Artifactual immunoreactivity of 1-3 of the antigens will not produce a positive test, therefore the test will be more robust and produce fewer false positives.

Identification Of CSF-Enriched Proteins:

[0031] CSF samples from 1-40 individuals are pooled and 200 μ l of the pooled samples are analyzed. Samples of sera from 1-40 individuals are pooled and 1 ml of pooled sera are analyzed. Major proteins shared by the blood and CSF (i.e. albumin, immunoglobins, etc.) were removed from both samples by repeated affinity chromatography.

[0032] In vitro label 50 μ g of the control protein extract and 50 μ g of the experimental protein extract with GE Healthcare Cy-3 and Cy-5 N-hydroxysuccinimidyl ester dyes. These dyes have been matched with respect to charge and mass - with the single positive charge of the dye replacing the charge lost by the modified lysine or N-terminus of the protein. Cy-3 and Cy-5 labeled proteins co-migrate - with the dye label adding approximately 450 Da to the proteins in each sample.

[0033] Control, experimental, and internal standard samples were mixed together (i.e., 150 μ g total protein) and then an equal volume of 2X Sample Buffer added.

[0034] The volume was brought up to 450 μ l with Rehydration Buffer. Immobiline™ (IPG) Drystrips (GE Healthcare) 24 cm were rehydrated for 10-24 hrs, and isoelectric focusing carried out. We used a number of different pH ranges including: 3-7, 4-7, 3.5-4.5, 4.0-5.0, 4.5-5.5, 5.0-6.0, 5.5-6.7, and 6-9. SDS polyacrylamide gel electrophoresis (second) dimension was carried out on a 10 inch wide by 7.5 inch tall by 1.0mm thick gel with one side coated with Gelbond®. We used a 12.5% polyacrylamide gel which will optimally separate 12-100 kD proteins.

[0035] Immediately after SDS PAGE, the gel (which is still held between two glass plates) was scanned at all 3 wavelengths simultaneously on the GE Healthcare Typhoon™ 9410 Imager. After scanning, 16 bit TIFF files of each color channel were exported for image analysis using the differential in-gel analysis module of the GE Healthcare DeCyder software package. After spot detection (which includes automatic background correction, spot volume normalization

and volume ratio calculation), a user defined "dust filter" was applied to each gel. This has the effect of automatically removing non-protein spot features from the gel and is followed by recalculation of experimental parameters.

[0036] The front glass plate was removed and the gel was then fixed and stained with Sypro Ruby, which is the fluorescent stain that was used as a guide to excise spots of interest from the gel. The reason for using Sypro Ruby, which stains all protein in the gel, is that the Cy-dye labeling is carried out such that the extent of incorporation will be <5% in terms of mole Cy-dye/mole protein. Since the Cy-dye has a MW of about 580 Da, low MW proteins (e.g., 10 Kd) labeled with Cy-dyes will not exactly co-migrate in the SDS PAGE dimension with their non-labeled counterparts.

[0037] GE Healthcare DeCyder™ software was used to quantify the gel image and to identify a "pick list" of differentially expressed protein spots to be excised and subjected to MS-based protein identification. The DeCyder™ software can analyze any two Cy-dyed gel images, either on the same gel or on different gels, match the spots between the two images, and then identify differentially expressed protein spots. The DeCyder™ software automatically outputs a listing of statistically significant differences in protein expression including t-test values, using the Cy-2 internal standard. Differentially expressed spots were identified using a number of criteria including area, volume, 3D peak slope, 3D peak height, and/or statistical variation. Protein spots that show different degrees of intensity between the two samples were highlighted by the software and confirmed manually. The DeCyder™ software was also used to analyze Sypro Ruby images, match the spots found with Sypro staining to those identified with the Cy-dye stains, and then choose a 'pick list' from the Sypro stained gel image.

[0038] The protein spot pick list was transferred to the Ettan™ Spot Picker instrument (GE Healthcare) which automatically excised the selected protein spots from the gel and transferred them into a 96-well microtiter plate.

[0039] The excised protein spots were then subjected to automated in-gel tryptic digestion on the Ettan™ TA Digester.

[0040] An aliquot of each digest was spotted (along with matrix) onto a MALDI-MS target.

[0041] High mass accuracy, automated MALDI-MS/MS spectra were acquired on each target (using an Applied Biosystems 4800 Tof/Tof instrument) and the resulting peptide masses were subjected to database searching using Mascot algorithms.

[0042] The remaining aliquots of digests of protein spots that are not identified by this approach were subjected to nanospray or LC/MS/MS analysis (Micromass Q-Tof) with the resulting MS/MS spectra then being subjected to Sequest database searches to identify proteins present in the sample.

CSF-enriched protein phosphorylation sites as antigens for a CSF test strip:

[0043] During the course of Fluorescence Difference Gel Electrophoresis (DIGE) experiments to identify CSF-enriched proteins, spots distributed in the pH dimension that were highly CSF-enriched (i.e. not present in blood samples) were identified, however upon protein identification by LC-MS, it was established that many of these proteins were in fact present in the blood but had a different patterns in the pH dimension of the gel (Figure 6). Regularly spaced spots of the same molecular weight often represent differentially phosphorylated versions of the same protein. The differential and regular migration in the pH dimension is indicative of the large but quantal nature of the negative charge on the PO₃⁻ groups. Upon phosphopeptide mapping of these spot arrays, it was determined that this was in fact the case. Several of these proteins (including angiotensinogen, (Figure 6) had highly CSF-enriched phosphorylations. In some cases these phosphorylation sites were serine/threonine phosphorylations, and in other cases they were tyrosine phosphorylations. In all, proteins were selected with multiple CSF-enriched sites per protein (i.e. angiotensinogen). As it is possible to produce antibodies that recognize a single epitope only when phosphorylated, phosphorylation sites will be included as antigens in the assays described herein. These phosphorylated epitopes are attractive as candidates as they are very prevalent and the presence of two CSF-enriched phosphorylation sites on a single protein opens the door to making pairs of antibodies to different sites that can be used differentially on the mobile and immobile regions of the strip to require dual phosphorylation for a positive response. We have run DIGE gels comparing CSF proteins that have been dephosphorylated with alkaline phosphatase (Figure 9). This has identified proteins listed herein as differentially phosphorylated in the CSF.

[0044] Identification of antigens is performed using 2 dimensional DIGE gel electrophoresis followed by trypsin digestion and LC-MS. The dominant proteins in both blood and CSF are removed by affinity columns prior to electrophoresis. These proteins are ubiquitously present in bodily fluids (i.e. albumin, immunoglobins etc.). We run all samples doubly across columns to remove 14 dominant serum proteins. We run the extracted proteins from 1-2 mls of whole blood on gels along with proteins from 200 µl of CSF. This enriched the blood proteins to ensure we are identifying proteins that are enriched in the CSF. Proteins from the CSF are labeled using either Cy3 or Cy5 fluorophores. In contrast blood proteins are labeled with either Cy5 or Cy3, respectively. The samples are then mixed and loaded on a 2 dimensional PAGE gel. Numerous different gels are run focusing on different regions of the molecular mass dimension (Y-dimension) and pH dimension (X-dimension). Following running of the gel, the intensity of the differentially visualized fluorescently labeled proteins are quantified and compared by an automated computer program. Those spots that are enriched by at least 5 x in the CSF are robotically collected, trypsin digested and analyzed by LC-MS. Peptide molecular weights are

compared to published databases. Enriched proteins are selected as candidates and standard molecular biologic methodology are employed for the production of Histidine-tagged recombinant proteins in bacteria or alternatively peptides corresponding to specific regions of the proteins are produced synthetically. Monoclonal and polyclonal antibodies are produced by a commercial house using provided antigens. Affinity purification is performed by standard column techniques utilizing cyanogen bromide-activated columns and recombinant proteins used for immunization. CSF-specific antigens are identified by trypsin and chymotrypsin digestion followed by LC-MS and phosphopeptide determination.

[0045] Validation of CSF-enriched antibodies is conducted by separating discrete volumes of whole bodily fluid proteins on SDS-PAGE, transferring to nitrocellulose membranes, immunoblotting first with primary antibodies against the antigens and then HRP-labeled secondary antibodies followed by ECL quantification. Antigens that have a >5x immunoreactivity in CSF over levels larger volumes of whole blood, nasal and ear effluents, tear, saliva or sweat are pursued. Samples of bodily fluids from 20 to 30 different individuals of each are tested for each antigen. Fluid samples are purchased from commercial laboratories that assure purity or directly collected. Bodily fluids are tested from individuals ranging in age from infants to elderly (75 years), male and female, as well as several common pathological conditions (i.e. advanced stage diabetes, coronary artery disease, asthma, etc.).

[0046] To identify phosphorylation state specific antigens, two-dimensional gels are produced as described above however three labeled protein fractions are produced (Cy2, Cy3 and Cy5): CSF, whole blood and CSF proteins in which all protein phosphorylations have been removed by alkaline phosphatase in an additional step prior to labeling. A comparison is then made between the dephosphorylated and normal CSF channels for alterations. Spots that disappear following dephosphorylation and are not present in the blood protein fluorescence channel are collected and sequenced. Absolute identification of the site of phosphorylation is determined by phospho peptide and phospho amino acid analysis, *in vitro* phosphorylation of recombinant proteins and protein fragments and immunoreactivity with phosphostate specific antibodies.

[0047] Once antibodies have been selected for use in the test strips, the relative affinity of each of the antibodies will be determined by running dilution curves using pure samples of recombinant antigens. This will guide the mixing of antibodies for inclusion on test strips.

[0048] In one embodiment, included herein are the use of devices and methods for rapid, bedside or triage site testing of bodily fluids, surgical sites or wounds for the presence of cerebrospinal fluid. In another embodiment a test is proposed that allows detection of CSF enriched proteins in samples of blood, plasma or sera as an indication of central nervous system (CNS) injury, breach or damage. Tests can include a single or multiples of the antigens described herein in addition to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof as markers of damage to the CNS. Described herein are newly-identified CSF-specific or enriched antigens that can be used individually or in combination in addition to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof to detect CSF in a broad spectrum of individuals from pediatric to geriatric, and despite the presence of diseases, personal habits, or individual genetic variability that might alter the composition of bodily fluids.

[0049] In one embodiment, included herein is the use of devices for the detection of cerebrospinal fluid in samples such as those suspected of containing cerebrospinal fluid, wherein the devices can include in addition to an antibody to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof one or more antibodies specific for one or more CSF antigens as described above. The CSF antigens can be employed in combinations to enhance the signal to noise ratio and to overcome individual variability in the expression of the antigens described above in different bodily fluids. In some embodiments, the detection of multiple antigens provides superior sensitivity and selectivity over detection of a single CSF-enriched antigen.

[0050] In one embodiment, described herein is the use of devices for the detection of cerebrospinal fluid in samples such as those suspected of containing cerebrospinal fluid, wherein the devices can include, in addition to an antibody to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof, one or more antibodies specific for one or more CSF antigens in a state of post-translational modification that is specific to the cerebrospinal fluid and distinguishable from the same antigen in other bodily fluids by virtue of the post-translational modification.

[0051] In some embodiments, described herein is the use of devices for the detection of cerebrospinal fluid in samples such as those suspected of containing cerebrospinal fluid, wherein the devices can include, in addition to an antibody to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof, one or more antibodies specific for one or more CSF antigens in a state of phosphorylation that is specific to the cerebrospinal fluid and distinguishable from the same antigen in other bodily fluids by virtue of the phosphorylation.

[0052] Samples for testing using the devices disclosed herein can be taken from different sites in the human body, such as at a site of surgery (i.e. head, neck, ear, throat, nasal or spinal surgeries) where the potential for CSF leakage

is possible; at a site of epidural injection or spinal tap; or at a site of wounds in areas where a breach of the meninges is possible (i.e. head, neck, spinal cord, nasal compartment, nose, ears, throat, skull, etc.), or where the injured party demonstrates signs of possible meningeal breach or serious injury to the central nervous system; or at a site of epidural injection, spinal injection or spinal tap. The antigens identified herein are particularly good markers for brain injury.

Additional samples include saliva and urine samples.

[0053] The unique approach of performing 2D-DIGE studies to compare the components of human CSF and serum has yielded a number of antigens that are specific to, or

highly enriched in CSF. Antibodies specific for these antigens are markers of the presence of CSF in bodily fluids, or at wound, surgical or injections sites where its presence would be atypical and potentially threaten the health or life of a patient or trauma victim.

[0054] In some embodiments, the above-described CSF antigens have post-translational modifications such as phosphorylation, glycosylation, sumoylation, ubiquitination, lipidation, nitrosylation, acetylation, neddylation, where those post-translational modification are specific to the CSF form of the antigen may be used by the lateral flow assay, western blots, ELISA or immunoprecipitation.

[0055] In some embodiments, multiple antigens may be used including cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof and may include combinations of antibodies that detect simple antigens (i.e., unmodified antigens) with antibodies that detect post-translationally modified antigens such as described above and in any of the various assays, lateral flow, Western blot, ELISA, or immunoprecipitation.

[0056] In one embodiment, antibodies are used to determine if a sample contains, in addition to cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof polypeptides associated with the presence of CSF indicating the presence or absence of CSF. Antibody binding is detected by, for example, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, surface plasmon resonance, immunocytochemistry, immunohistochemistry, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (e.g., using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, and the like. Detection of antibody binding can be achieved using enzymatic, colorimetric, fluorescent, bioluminescent, luminescent, colored latex beads, colloidal gold and/or silver methods.

[0057] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting binding in an immunoassay.

[0058] In some embodiments, an automated detection assay is utilized. Methods for the automation of immunoassays include those described in U.S. Pat. Nos. 5,885,530, 4,981,785, 6,159,750, and 5,358,691. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, software that generates a score correlating to the presence of specific polypeptides and likelihood of CSF in a sample based on the result of the immunoassay is utilized.

[0059] In other embodiments, the immunoassay is as described in U.S. Pat. Nos. 5,599,677 and 5,672,480.

[0060] Provided herein are isolated antibodies or antibody fragments (e.g., Fab fragments, Fab₂ fragments, and the like). Antibodies can be generated to allow for the detection of polypeptides associated with the presence of CSF. The antibodies are prepared using various polypeptides, synthetic peptides and/or recombinant proteins associated with the presence of CSF and fragments thereof. In one embodiment, the immunogens used to generate antibodies that recognize the polypeptides associated with the presence of CSF are polypeptides, synthetic peptides and/or recombinant proteins associated with the presence of CSF. In one embodiment, the antibody is reactive with a native or "folded" protein. In another embodiment, an antibody is reactive with denatured protein (including detergent solubilized). Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, Fab expression libraries, or recombinant (e.g., chimeric, humanized, etc.) antibodies, as long as it can recognize the protein. Antibodies can be produced by using a protein or peptide as the antigen according to a conventional antibody or antiserum preparation process.

[0061] Various procedures are used for the production of polyclonal antibodies directed against polypeptides associated with the presence of CSF. For the production of an antibody, various host animals are immunized by injection with the polypeptides, synthetic peptides and/or recombinant proteins associated with the presence of CSF or a fragment thereof including but not limited to rabbits, mice, rats, sheep, goats, chicken, donkey, etc. In a specific instance, the peptide is conjugated to an immunogenic carrier (e.g., diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins,

dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum).

5 [0062] For preparation of monoclonal antibodies directed toward polypeptides, synthetic peptides and recombinant proteins associated with the presence of CSF, it is contemplated that a technique that provides for the production of antibody molecules by continuous cell lines in culture will find use herein. These include, but are not limited to, the hybridoma technique originally developed by Kohler and Milstein, as well as the trioma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique to produce human monoclonal antibodies.

10 [0063] In additional instances, monoclonal antibodies are produced in germ-free animals. Furthermore, it is contemplated that human antibodies will be generated by human hybridomas or by transforming human B cells with EBV virus in vitro.

[0064] In addition, it is contemplated that techniques described for the production of single chain antibodies will find use in producing single chain antibodies. An additional embodiment utilizes the techniques described for the construction of Fab expression libraries to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

15 [0065] In other embodiments, contemplated are the use of recombinant antibodies or fragments thereof to polypeptides associated with the presence of CSF. Recombinant antibodies include, but are not limited to, humanized and chimeric antibodies. Methods for generating recombinant antibodies are known in the art.

20 [0066] It is contemplated that a technique suitable for producing antibody fragments will find use in generating antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule. For example, such fragments include but are not limited to: F(ab')₂ fragment that can be produced by pepsin digestion of the antibody molecule; Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent.

25 [0067] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. The immunogenic peptide may be provided free of the carrier molecule used in any immunization protocol. For example, if the peptide was conjugated to KLH, it may be conjugated to BSA, or used directly, in a screening assay.

30 [0068] The foregoing antibodies can be used in methods known in the art relating to the localization and structure of polypeptides associated with the presence of CSF (e.g., for Western blotting), measuring levels thereof in appropriate biological samples, etc. The antibodies can be used to detect polypeptides associated with the presence of CSF in a biological sample from an individual. The biological sample is a biological fluid, such as, but not limited to, tissue, blood, serum, plasma, urine, nasal and ear effluents, saliva, sweat, tears and the like. In one embodiment, the sample is from an individual suspected of having a brain injury, such as mild traumatic head injury received during participation in sporting events, auto accidents, military activity and motorcycle accidents. The test would be most useful when the injury is mild to moderate in severity. More severe head injury including penetrating injuries generally already receive the necessary level of

35 medical attention. Diagnosis of traumatic brain injuries generally requires a short neurological exam (the GCS). The precise designations of mild and moderate are sometimes hard to objectively identify without a recent baseline, pre injury test. Other injuries or treatments (sedative, anesthetics, etc) can interfere with the test. The current set of antigens can represent "biomarkers" which could be used to "fingerprint" the existence and severity of a head injury. A rapid test that is qualitative or quantitative of the existence of a subset of these antigens in blood or other bodily fluids (sweat, urine, saliva, etc.) can be used as a measure of the severity of an injury in combination with a GCS or any such neurological exam. Often the severity of a mild to moderate head injury is not know and to what degree the person should continue to engage in critical activities (i.e. continuing to participate in a sporting event, continue to work or drive a vehicle, remain in the combat arena, continue to assume a command position in combat, operate heavy machinery, etc.). A more objective test of blood borne or secreted proteins normally found enriched only in the CSF would represent a diagnostic test of injury.

40 [0069] The biological samples can then be tested directly for the presence of polypeptides associated with the presence of CSF using an appropriate strategy (e.g., ELISA or radioimmunoassay) and format (e.g., microwells, dipstick (e.g., as described in International Patent Publication WO 93/03367), etc. Alternatively, proteins in the sample can be size separated (e.g., by polyacrylamide gel electrophoresis (PAGE), in the presence or not of sodium dodecyl sulfate (SDS) Triton, Nonidet (or other ionic or non-ionic detergents), and the presence of a CSF antigen detected by immunoblotting (Western blotting). Immunoblotting techniques are generally more effective with antibodies generated against a peptide corresponding to an epitope of a protein, and hence, are particularly suited to the present disclosure.

45 [0070] The correlation step mentioned above may be implemented qualitatively or quantitatively, for example in a fluorophoric or colorimetric assay.

Kits and devices:

[0071] Also described herein are kits and devices for determining whether a sample contains polypeptides associated with the presence of CSF. The diagnostic kits and devices are produced in a variety of ways. In some instances, the kits and devices contain at least one reagent for specifically detecting a polypeptide associated with the presence of CSF. In more specific instances, the kits and devices contain multiple reagents for detecting polypeptides associated with the presence of CSF. In other instances, the reagents are antibodies that preferentially bind polypeptides associated with the presence of CSF. The test can produce a single result indicating the presence of CSF from a number (2-10) of tests for multiple antigens or each test can produce a different evident result that can be interpreted to indicate the presence or absence of CSF.

[0072] In some instances, the kit or device contains instructions for determining whether the sample contains polypeptides associated with the presence of CSF. In specific instances, the instructions specify that presence or absence of CSF is determined by detecting the presence or absence of polypeptides associated with the presence of CSF in a sample from the subject.

[0073] In some instances, the kits and devices include ancillary reagents such as buffering agents, protein stabilizing reagents, and signal producing systems (e.g., fluorescence generating systems such as FRET systems). The test kit or device is packaged in a 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 instances, the kits or devices also include a positive control sample. In further instances, the kit or device contains comparative reference material to interpret the presence or absence of polypeptides associated with the presence of CSF according to intensity, color spectrum, or other physical attribute of an indicator.

[0074] The need for a rapid, reproducible, sensitive and simple diagnostic test, which can be used in the health care for diagnosing CSF, is of major importance. Such a test has the obvious advantage over the existing laboratory tests, i.e., immunofixation electrophoresis, enzyme-linked immunosorbant assay (ELISA) and immunoblotting, in that it can be performed immediately beside the patient giving a result in a few minutes of time instead of several days when the sample is sent for analysis to a laboratory. A lateral flow immunochromatographic test may be utilized for making a diagnostic kit for the detection of CSF in biological fluids.

[0075] In one instance, a device includes a solid phase comprising a first region comprising a mobile indicator suitable for binding a CSF antigen, and a second region comprising a fixed indicator suitable for binding the CSF antigen.

[0076] In one instance, a lateral flow device comprises a test strip optionally with a plastic test cassette. Antibodies are attached to three different zones on the membrane; a sample zone (S) containing a first monoclonal antibody to a polypeptide associated with the presence of CSF; a test zone (T) that contains a second monoclonal or polyclonal antibody to polypeptides associated with the presence of CSF immobilized to the membrane; and a control zone (C), which contains a control antibody, for example, an immobilized rabbit anti-mouse antibody. The first monoclonal antibody in the sample (S) zone may be conjugated to a mobile particle, for example, a colored latex particle or a gold particle. Alternatively, the first monoclonal antibody is conjugated to a chromophoric indicator, such as a fluorescent molecule or tag (Green Fluorescent Protein (GFP) or FP orthologs mutants and other naturally occurring GFP-like fluorescent and chromo proteins, fluorescein (and orthologs), rhodamine (and orthologs), Cy3, Cy5, Cy2, Cy7, Cy8, Alexa® dyes, Texas Red, and the like).

[0077] An exemplary device is implemented utilizing an immunochromatographic test based on the use of two monoclonal antibodies. Sample is added to the S-zone, and if the polypeptide associated with the presence of CSF is present, it binds to the first monoclonal antibody to form a polypeptide-conjugate-complex. This complex migrates chromatographically on the membrane, and when it reaches the immobilized antibody in the T-zone, agglutination takes place and a blue colored band is formed.

[0078] Briefly and in one instance, the first monoclonal antibody is conjugated to a mobile particle, for example, gold or latex beads. These beads have the intrinsic color of either being red (for gold) or can come in different colors if using latex beads. When the sample is applied on the "S-zone", the marker, a polypeptides associated with the presence of CSF if present in the sample, binds to the first monoclonal antibody that is conjugated to the beads and then because of the lateral flow absorbent pad on which the beads are placed, the complex (beads+antibody+polypeptide if present in the sample) migrates laterally. Once the complex reaches the "T-zone" where the second antibody is immobilized on the strip, the marker that is now migrating with the complex binds to the second immobilized antibody. As the second antibody is stationary/fixed/immobilized, the whole complex gets trapped and as the complex now contains colored beads, the immobilized T-zone line lights up according to the beads that are used (red for gold or different colors {like blue} if latex beads are used). The excess complex sample migrates to the end of the strip and at the "C-zone" the first antibody conjugated to the beads is trapped by immobilized/fixed/stationary rabbit-anti mouse antibody and gives a colored line indicating that the test is complete). Thus, a colored band indicates a positive result. No band in the T-zone is significant for a negative result. The immobilized polyclonal antibody in the C-zone will bind the latex conjugate with both positive and negative samples. This blue band assures a correct test performance.

[0079] In practice, the kits and devices are utilized in a variety of clinical settings to determine the presence of CSF in a sample.

[0080] The invention is illustrated by the following non-limiting examples. Examples:

[0081] CSF-specific antigens newly identified herein include Isoform 1 of Neural cell adhesion molecule-like (Accession Number gi|62088238) protein; Chain A, Human Mesotrypsin Complexed With Bovine Pancreatic Trypsin Inhibitor(Bpti) (Accession number gi|162330095); CNTN2 Contactin-2 (Accession Number gi|4827022); CNTN1 Isoform 2 of Contactin-1 (Accession Number gi:28373119); cDNA highly similar to SPARC-like protein 1 (Accession Number: gi|194388050); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (Accession Number: gi|68534652 and gi|109731501); NCAM2 Neural cell adhesion molecule 2 (Accession Number gi|119630409); SERPINA3 serpin peptidase inhibitor, clade A, member 3 precursor /Isoform 1 of Alpha-1-antichymotrypsin/growth-inhibiting protein 25 [Homo sapiens] or slightly longer fragment of alpha-1-antichymotrypsin precursor (Accession Number gi|46981961); AGT Angiotensinogen (Accession Number gi|553181); Angiotensinogen precursor (Serpin A8) (Accession Number gi|4557287); unnamed protein product also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3; possible fragment (Accession Number gi|187608363); cDNA FLJ59893, dickkopf homolog 3 precursor (Accession Number gi|40548389); SERPINF1 serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 isoform 4 [Pan troglodytes] factor (Accession Number gi|15988024); GC Vitamin D-binding protein PREDICTED: vitamin D-binding protein [Pan troglodytes] (Accession Number 181482); CD14 Human monocyte antigen CD14 (CD14) (Accession Number gi|117646212); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (Accession Number gi|90080503; gi|187608363 (human)); Neural cell adhesion molecule variant (Accession Number gi62088238); CLU cDNA FLJ57622, highly similar to Clusterin (Accession number gi|189054091); protein highly similar to Clusterin (Accession number gi|193787502); LMAN2 Vesicular integral-membrane protein VIP36 (Accession number gi|157834800); superoxide dismutase 3, extracellular precursor (Accession number gi|18582275); fibrin alpha C term fragment (Accession number gi|223057); KLK6 Isoform 1 of Kallikrein-6 (Accession number gi|21465970); APCS Serum amyloid P-component/Chain A, The Structure Of Pentameric Human Serum Amyloid P Component (Accession number gi|576259); FAM3C Protein FAM3C / family with sequence similarity 3, member C precursor [Homo sapiens] note="predicted osteoblast protein; interleukin-like EMT inducer (Accession number gi|55629272); Chain A, Human Kallikrein 6 (Hk6) Active Form With Benzamidine Inhibitor (Accession number gi|21465970); unnamed protein product [Macaca fascicularis] also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3; possible fragment (Accession number gi|187608363); a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF antigens; or a combination of two or more of the foregoing CSF antigens.

[0082] The terms "a" and "an" herein do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item.

[0083] All ranges disclosed herein are inclusive and combinable. While the invention has been described with reference to a preferred embodiment, it will be understood by those skilled in the art that it is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the appended claims.

SEQUENCE LISTING

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<110> Pieribone, Vincent

<120> DEVICE AND METHODS FOR THE IMMUNOLOGICAL IDENTIFICATION OF CEREBROSPINAL FLUID

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<150> US 61/232,033

<151> 2009-08-07

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EP 2 462 444 B1

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 405 410 415
 55

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Asn Ile Asp Val Val Asp Val Arg Pro Leu Ile Gln Thr Lys Asp Gly
 420 425 430
 5 Glu Asn Tyr Ala Thr Val Val Gly Tyr Ser Ala Phe Leu His Cys Glu
 435 440 445
 10 Phe Phe Ala Ser Pro Glu Ala Val Val Ser Trp Gln Lys Val Glu Glu
 450 455 460
 Val Lys Pro Leu Glu Gly Arg Arg Tyr His Ile Tyr Glu Asn Gly Thr
 465 470 475 480
 15 Leu Gln Ile Asn Arg Thr Thr Glu Glu Asp Ala Gly Ser Tyr Ser Cys
 485 490 495
 20 Trp Val Glu Asn Ala Ile Gly Lys Thr Ala Val Thr Ala Asn Leu Asp
 500 505 510
 Ile Arg Asn Ala Thr Lys Leu Arg Val Ser Pro Lys Asn Pro Arg Ile
 515 520 525
 25 Pro Lys Leu His Met Leu Glu Leu His Cys Glu Ser Lys Cys Asp Ser
 530 535 540
 30 His Leu Lys His Ser Leu Lys Leu Ser Trp Ser Lys Asp Gly Glu Ala
 545 550 555 560
 Phe Glu Ile Asn Gly Thr Glu Asp Gly Arg Ile Ile Ile Asp Gly Ala
 565 570 575
 35 Asn Leu Thr Ile Ser Asn Val Thr Leu Glu Asp Gln Gly Ile Tyr Cys
 580 585 590
 40 Cys Ser Ala His Thr Ala Leu Asp Ser Ala Ala Asp Ile Thr Gln Val
 595 600 605
 Thr Val Leu Asp Val Pro Asp Pro Pro Glu Asn Leu His Leu Ser Glu
 610 615 620
 45 Arg Gln Asn Arg Ser Val Arg Leu Thr Trp Glu Ala Gly Ala Asp His
 625 630 635 640
 50 Asn Ser Asn Ile Ser Glu Tyr Ile Val Glu Phe Glu Gly Asn Lys Glu
 645 650 655
 Glu Pro Gly Arg Trp Glu Glu Leu Thr Arg Val Gln Gly Lys Lys Thr
 660 665 670
 55

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Thr Val Ile Leu Pro Leu Ala Pro Phe Val Arg Tyr Gln Phe Arg Val
 675 680 685
 5 Ile Ala Val Asn Glu Val Gly Arg Ser Gln Pro Ser Gln Pro Ser Asp
 690 695 700
 His His Glu Thr Pro Pro Ala Ala Pro Asp Arg Asn Pro Gln Asn Ile
 705 710 715 720
 10 Arg Val Gln Ala Ser Gln Pro Lys Glu Met Ile Ile Lys Trp Glu Pro
 725 730 735
 15 Leu Lys Ser Met Glu Gln Asn Gly Pro Gly Leu Glu Tyr Arg Val Thr
 740 745 750
 Trp Lys Pro Gln Gly Ala Pro Val Glu Trp Glu Glu Glu Thr Val Thr
 755 760 765
 20 Asn His Thr Leu Arg Val Met Thr Pro Ala Val Tyr Ala Pro Tyr Asp
 770 775 780
 25 Val Lys Val Gln Ala Ile Asn Gln Leu Gly Ser Gly Pro Asp Pro Gln
 785 790 795 800
 Ser Val Thr Leu Tyr Ser Gly Glu Asp Tyr Pro Asp Thr Ala Pro Val
 805 810 815
 30 Ile His Gly Val Asp Val Ile Asn Ser Thr Leu Val Lys Val Thr Trp
 820 825 830
 35 Ser Thr Val Pro Lys Asp Arg Val His Gly Arg Leu Lys Gly Tyr Gln
 835 840 845
 40 Ile Asn Trp Trp Lys Thr Lys Ser Leu Leu Asp Gly Arg Thr His Pro
 850 855 860
 Lys Glu Val Asn Ile Leu Arg Phe Ser Gly Gln Arg Asn Ser Gly Met
 865 870 875 880
 45 Val Pro Ser Leu Asp Ala Phe Ser Glu Phe His Leu Thr Val Leu Ala
 885 890 895
 Tyr Asn Ser Lys Gly Ala Gly Pro Glu Ser Glu Pro Tyr Ile Phe Gln
 900 905 910
 50 Thr Pro Glu Gly Val Pro Glu Gln Pro Thr Phe Leu Lys Val Ile Lys
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		915				920					925					
5	Val	Asp 930	Lys	Asp	Thr	Ala	Thr 935	Leu	Ser	Trp	Gly	Leu 940	Pro	Lys	Lys	Leu
	Asn	Gly	Asn	Leu	Thr	Gly 950	Tyr	Leu	Leu	Gln	Tyr 955	Gln	Ile	Ile	Asn	Asp 960
10	Thr	Tyr	Glu	Ile	Gly 965	Glu	Leu	Asn	Asp	Ile 970	Asn	Ile	Thr	Thr	Pro	Ser 975
	Lys	Pro	Ser	Trp 980	His	Leu	Ser	Asn	Leu 985	Asn	Ala	Thr	Thr	Lys 990	Tyr	Lys
	Phe	Tyr	Leu 995	Arg	Ala	Cys	Thr	Ser 1000	Gln	Gly	Cys	Gly	Lys 1005	Pro	Ile	Thr
20	Glu	Glu 1010	Ser	Ser	Thr	Leu	Gly 1015	Glu	Gly	Ser	Lys	Gly 1020	Ile	Gly	Lys	
	Ile	Ser 1025	Gly	Val	Asn	Leu	Thr 1030	Gln	Lys	Thr	His	Pro 1035	Val	Glu	Val	
25	Phe	Glu 1040	Pro	Gly	Ala	Glu	His 1045	Ile	Val	Arg	Leu	Met 1050	Thr	Lys	Asn	
30	Trp	Gly 1055	Asp	Asn	Asp	Ser	Ile 1060	Phe	Gln	Asp	Val	Ile 1065	Glu	Thr	Arg	
	Gly	Arg 1070	Glu	Tyr	Ala	Gly	Leu 1075	Tyr	Asp	Asp	Ile	Ser 1080	Thr	Gln	Gly	
35	Trp	Phe 1085	Ile	Gly	Leu	Met	Cys 1090	Ala	Ile	Ala	Leu	Leu 1095	Thr	Leu	Leu	
40	Leu	Leu 1100	Thr	Val	Cys	Phe	Val 1105	Lys	Arg	Asn	Arg	Gly 1110	Gly	Lys	Tyr	
	Ser	Val 1115	Lys	Glu	Lys	Glu	Asp 1120	Leu	His	Pro	Asp	Pro 1125	Glu	Ile	Gln	
45	Ser	Val 1130	Lys	Asp	Glu	Thr	Phe 1135	Gly	Glu	Tyr	Ser	Asp 1140	Ser	Asp	Glu	
50	Lys	Pro 1145	Leu	Lys	Gly	Ser	Leu 1150	Arg	Ser	Leu	Asn	Arg 1155	Asp	Met	Gln	
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Pro Thr Glu Ser Ala Asp Ser Leu Val Glu Tyr Gly Glu Gly Asp
 1160 1165 1170
 5 His Gly Leu Phe Ser Glu Asp Gly Ser Phe Ile Gly Ala Tyr Ala
 1175 1180 1185
 10 Gly Ser Lys Glu Lys Gly Ser Val Glu Ser Asn Gly Ser Ser Thr
 1190 1195 1200
 Ala Thr Phe Pro Leu Arg Ala
 1205 1210
 15 <210> 2
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 <213> Homo sapiens
 20 <400> 2
 Ile Val Gly Gly Tyr Thr Cys Glu Glu Asn Ser Leu Pro Tyr Gln Val
 1 5 10 15
 25 Ser Leu Asn Ser Gly Ser His Phe Cys Gly Gly Ser Leu Ile Ser Glu
 20 25 30
 30 Gln Trp Val Val Ser Ala Ala His Cys Tyr Lys Thr Arg Ile Gln Val
 35 40 45
 Arg Leu Gly Glu His Asn Ile Lys Val Leu Glu Gly Asn Glu Gln Phe
 50 55 60
 35 Ile Asn Ala Ala Lys Ile Ile Arg His Pro Lys Tyr Asn Arg Asp Thr
 65 70 75 80
 40 Leu Asp Asn Asp Ile Met Leu Ile Lys Leu Ser Ser Pro Ala Val Ile
 85 90 95
 45 Asn Ala Arg Val Ser Thr Ile Ser Leu Pro Thr Ala Pro Pro Ala Ala
 100 105 110
 50 Gly Thr Glu Cys Leu Ile Ser Gly Trp Gly Asn Thr Leu Ser Phe Gly
 115 120 125
 Ala Asp Tyr Pro Asp Glu Leu Lys Cys Leu Asp Ala Pro Val Leu Thr
 130 135 140
 55 Gln Ala Glu Cys Lys Ala Ser Tyr Pro Gly Lys Ile Thr Asn Ser Met
 145 150 155 160

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Phe Cys Val Gly Phe Leu Glu Gly Gly Lys Asp Ser Cys Gln Arg Asp
 165 170 175
 5 Ala Gly Gly Pro Val Val Cys Asn Gly Gln Leu Gln Gly Val Val Ser
 180 185 190
 10 Trp Gly His Gly Cys Ala Trp Lys Asn Arg Pro Gly Val Tyr Thr Lys
 195 200 205
 Val Tyr Asn Tyr Val Asp Trp Ile Lys Asp Thr Ile Ala Ala Asn Ser
 210 215 220
 15 <210> 3
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 <212> PRT
 <213> homo sapiens
 20 <400> 3
 Met Gly Thr Ala Thr Arg Arg Lys Pro His Leu Leu Leu Val Ala Ala
 1 5 10
 25 Val Ala Leu Val Ser Ser Ser Ala Trp Ser Ser Ala Leu Gly Ser Gln
 20 25 30
 30 Thr Thr Phe Gly Pro Val Phe Glu Asp Gln Pro Leu Ser Val Leu Phe
 35 40 45
 Pro Glu Glu Ser Thr Glu Glu Gln Val Leu Leu Ala Cys Arg Ala Arg
 50 55 60
 35 Ala Ser Pro Pro Ala Thr Tyr Arg Trp Lys Met Asn Gly Thr Glu Met
 65 70 75 80
 40 Lys Leu Glu Pro Gly Ser Arg His Gln Leu Val Gly Gly Asn Leu Val
 85 90 95
 45 Ile Met Asn Pro Thr Lys Ala Gln Asp Ala Gly Val Tyr Gln Cys Leu
 100 105 110
 Ala Ser Asn Pro Val Gly Thr Val Val Ser Arg Glu Ala Ile Leu Arg
 115 120 125
 50 Phe Gly Phe Leu Gln Glu Phe Ser Lys Glu Glu Arg Asp Pro Val Lys
 130 135 140
 Ala His Glu Gly Trp Gly Val Met Leu Pro Cys Asn Pro Pro Ala His
 145 150 155 160
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Tyr Pro Gly Leu Ser Tyr Arg Trp Leu Leu Asn Glu Phe Pro Asn Phe
 165 170 175
 5 Ile Pro Thr Asp Gly Arg His Phe Val Ser Gln Thr Thr Gly Asn Leu
 180 185 190
 Tyr Ile Ala Arg Thr Asn Ala Ser Asp Leu Gly Asn Tyr Ser Cys Leu
 10 195 200 205
 Ala Thr Ser His Met Asp Phe Ser Thr Lys Ser Val Phe Ser Lys Phe
 210 215 220
 15 Ala Gln Leu Asn Leu Ala Ala Glu Asp Thr Arg Leu Phe Ala Pro Ser
 225 230 235 240
 Ile Lys Ala Arg Phe Pro Ala Glu Thr Tyr Ala Leu Val Gly Gln Gln
 20 245 250 255
 Val Thr Leu Glu Cys Phe Ala Phe Gly Asn Pro Val Pro Arg Ile Lys
 260 265 270
 25 Trp Arg Lys Val Asp Gly Ser Leu Ser Pro Gln Trp Thr Thr Ala Glu
 275 280 285
 Pro Thr Leu Gln Ile Pro Ser Val Ser Phe Glu Asp Glu Gly Thr Tyr
 30 290 295 300
 Glu Cys Glu Ala Glu Asn Ser Lys Gly Arg Asp Thr Val Gln Gly Arg
 305 310 315 320
 35 Ile Ile Val Gln Ala Gln Pro Glu Trp Leu Lys Val Ile Ser Asp Thr
 325 330 335
 Glu Ala Asp Ile Gly Ser Asn Leu Arg Trp Gly Cys Ala Ala Ala Gly
 40 340 345 350
 Lys Pro Arg Pro Thr Val Arg Trp Leu Arg Asn Gly Glu Pro Leu Ala
 355 360 365
 45 Ser Gln Asn Arg Val Glu Val Leu Ala Gly Asp Leu Arg Phe Ser Lys
 370 375 380
 Leu Ser Leu Glu Asp Ser Gly Met Tyr Gln Cys Val Ala Glu Asn Lys
 50 385 390 395 400
 His Gly Thr Ile Tyr Ala Ser Ala Glu Leu Ala Val Gln Ala Leu Ala
 405 410 415
 55

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Pro Asp Phe Arg Leu Asn Pro Val Arg Arg Leu Ile Pro Ala Ala Arg
 420 425 430

5 Gly Gly Glu Ile Leu Ile Pro Cys Gln Pro Arg Ala Ala Pro Lys Ala
 435 440 445

Val Val Leu Trp Ser Lys Gly Thr Glu Ile Leu Val Asn Ser Ser Arg
 450 455 460

10 Val Thr Val Thr Pro Asp Gly Thr Leu Ile Ile Arg Asn Ile Ser Arg
 465 470 475 480

15 Ser Asp Glu Gly Lys Tyr Thr Cys Phe Ala Glu Asn Phe Met Gly Lys
 485 490 495

Ala Asn Ser Thr Gly Ile Leu Ser Val Arg Asp Ala Thr Lys Ile Thr
 500 505 510

20 Leu Ala Pro Ser Ser Ala Asp Ile Asn Leu Gly Asp Asn Leu Thr Leu
 515 520 525

25 Gln Cys His Ala Ser His Asp Pro Thr Met Asp Leu Thr Phe Thr Trp
 530 535 540

Thr Leu Asp Asp Phe Pro Ile Asp Phe Asp Lys Pro Gly Gly His Tyr
 545 550 555 560

30 Arg Arg Thr Asn Val Lys Glu Thr Ile Gly Asp Leu Thr Ile Leu Asn
 565 570 575

35 Ala Gln Leu Arg His Gly Gly Lys Tyr Thr Cys Met Ala Gln Thr Val
 580 585 590

Val Asp Ser Ala Ser Lys Glu Ala Thr Val Leu Val Arg Gly Pro Pro
 595 600 605

40 Gly Pro Pro Gly Gly Val Val Val Arg Asp Ile Gly Asp Thr Thr Ile
 610 615 620

45 Gln Leu Ser Trp Ser Arg Gly Phe Asp Asn His Ser Pro Ile Ala Lys
 625 630 635 640

Tyr Thr Leu Gln Ala Arg Thr Pro Pro Ala Gly Lys Trp Lys Gln Val
 645 650 655

50 Arg Thr Asn Pro Ala Asn Ile Glu Gly Asn Ala Glu Thr Ala Gln Val
 660 665 670

55

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Leu Gly Leu Thr Pro Trp Met Asp Tyr Glu Phe Arg Val Ile Ala Ser
 675 680 685
 5 Asn Ile Leu Gly Thr Gly Glu Pro Ser Gly Pro Ser Ser Lys Ile Arg
 690 700
 Thr Arg Glu Ala Ala Pro Ser Val Ala Pro Ser Gly Leu Ser Gly Gly
 705 710 715 720
 10 Gly Gly Ala Pro Gly Glu Leu Ile Val Asn Trp Thr Pro Met Ser Arg
 725 730 735
 15 Glu Tyr Gln Asn Gly Asp Gly Phe Gly Tyr Leu Leu Ser Phe Arg Arg
 740 745
 Gln Gly Ser Thr His Trp Gln Thr Ala Arg Val Pro Gly Ala Asp Ala
 755 760 765
 20 Gln Tyr Phe Val Tyr Ser Asn Glu Ser Val Arg Pro Tyr Thr Pro Phe
 770 775 780
 25 Glu Val Lys Ile Arg Ser Tyr Asn Arg Arg Gly Asp Gly Pro Glu Ser
 785 790 800
 Leu Thr Ala Leu Val Tyr Ser Ala Glu Glu Glu Pro Arg Val Ala Pro
 805 810 815
 30 Thr Lys Val Trp Ala Lys Gly Val Ser Ser Ser Glu Met Asn Val Thr
 820 825 830
 35 Trp Glu Pro Val Gln Gln Asp Met Asn Gly Ile Leu Leu Gly Tyr Glu
 835 840 845
 Ile Arg Tyr Trp Lys Ala Gly Asp Lys Glu Ala Ala Ala Asp Arg Val
 850 855 860
 40 Arg Thr Ala Gly Leu Asp Thr Ser Ala Arg Val Ser Gly Leu His Pro
 865 870 875 880
 45 Asn Thr Lys Tyr His Val Thr Val Arg Ala Tyr Asn Arg Ala Gly Thr
 885 890 895
 Gly Pro Ala Ser Pro Ser Ala Asn Ala Thr Thr Met Lys Pro Pro Pro
 900 905 910
 50 Arg Arg Pro Pro Gly Asn Ile Ser Trp Thr Phe Ser Ser Ser Ser Leu
 55

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				85					90				95			
5	Asp	Ala	Gly	Ile 100	Tyr	Tyr	Cys	Leu	Ala 105	Ser	Asn	Asn	Tyr	Gly 110	Met	Val
	Arg	Ser	Thr 115	Glu	Ala	Thr	Leu	Ser 120	Phe	Gly	Tyr	Leu	Asp 125	Pro	Phe	Pro
10	Pro	Glu 130	Glu	Arg	Pro	Glu	Val 135	Arg	Val	Lys	Glu	Gly 140	Lys	Gly	Met	Val
15	Leu 145	Leu	Cys	Asp	Pro	Pro 150	Tyr	His	Phe	Pro	Asp 155	Asp	Leu	Ser	Tyr	Arg 160
	Trp	Leu	Leu	Asn	Glu 165	Phe	Pro	Val	Phe	Ile 170	Thr	Met	Asp	Lys	Arg 175	Arg
20	Phe	Val	Ser	Gln 180	Thr	Asn	Gly	Asn	Leu 185	Tyr	Ile	Ala	Asn	Val 190	Glu	Ala
25	Ser	Asp	Lys 195	Gly	Asn	Tyr	Ser	Cys 200	Phe	Val	Ser	Ser	Pro 205	Ser	Ile	Thr
	Lys	Ser 210	Val	Phe	Ser	Lys	Phe 215	Ile	Pro	Leu	Ile	Pro 220	Ile	Pro	Glu	Arg
30	Thr 225	Thr	Lys	Pro	Tyr	Pro 230	Ala	Asp	Ile	Val	Val 235	Gln	Phe	Lys	Asp	Val 240
35	Tyr	Ala	Leu	Met	Gly 245	Gln	Asn	Val	Thr	Leu 250	Glu	Cys	Phe	Ala	Leu 255	Gly
	Asn	Pro	Val	Pro 260	Asp	Ile	Arg	Trp	Arg 265	Lys	Val	Leu	Glu	Pro 270	Met	Pro
40	Ser	Thr	Ala 275	Glu	Ile	Ser	Thr	Ser 280	Gly	Ala	Val	Leu	Lys 285	Ile	Phe	Asn
45	Ile	Gln 290	Leu	Glu	Asp	Glu	Gly 295	Ile	Tyr	Glu	Cys	Glu 300	Ala	Glu	Asn	Ile
	Arg 305	Gly	Lys	Asp	Lys	His 310	Gln	Ala	Arg	Ile	Tyr 315	Val	Gln	Ala	Phe	Pro 320
50	Glu	Trp	Val	Glu	His 325	Ile	Asn	Asp	Thr	Glu 330	Val	Asp	Ile	Gly	Ser 335	Asp

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Leu Tyr Trp Pro Cys Val Ala Thr Gly Lys Pro Ile Pro Thr Ile Arg
340 345 350

5 Trp Leu Lys Asn Gly Tyr Ala Tyr His Lys Gly Glu Leu Arg Leu Tyr
355 360 365

10 Asp Val Thr Phe Glu Asn Ala Gly Met Tyr Gln Cys Ile Ala Glu Asn
370 375 380

15 Thr Tyr Gly Ala Ile Tyr Ala Asn Ala Glu Leu Lys Ile Leu Ala Leu
385 390 395 400

20 Ala Pro Thr Phe Glu Met Asn Pro Met Lys Lys Lys Ile Leu Ala Ala
405 410 415

Lys Gly Gly Arg Val Ile Ile Glu Cys Lys Pro Lys Ala Ala Pro Lys
420 425 430

25 Pro Lys Phe Ser Trp Ser Lys Gly Thr Glu Trp Leu Val Asn Ser Ser
435 440 445

Arg Ile Leu Ile Trp Glu Asp Gly Ser Leu Glu Ile Asn Asn Ile Thr
450 455 460

30 Arg Asn Asp Gly Gly Ile Tyr Thr Cys Phe Ala Glu Asn Asn Arg Gly
465 470 475 480

Lys Ala Asn Ser Thr Gly Thr Leu Val Ile Thr Asp Pro Thr Arg Ile
485 490 495

35 Ile Leu Ala Pro Ile Asn Ala Asp Ile Thr Val Gly Glu Asn Ala Thr
500 505 510

40 Met Gln Cys Ala Ala Ser Phe Asp Pro Ala Leu Asp Leu Thr Phe Val
515 520 525

Trp Ser Phe Asn Gly Tyr Val Ile Asp Phe Asn Lys Glu Asn Ile His
530 535 540

45 Tyr Gln Arg Asn Phe Met Leu Asp Ser Asn Gly Glu Leu Leu Ile Arg
545 550 555 560

50 Asn Ala Gln Leu Lys His Ala Gly Arg Tyr Thr Cys Thr Ala Gln Thr
565 570 575

Ile Val Asp Asn Ser Ser Ala Ser Ala Asp Leu Val Val Arg Gly Pro
580 585 590

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Pro Gly Pro Pro Gly Gly Leu Arg Ile Glu Asp Ile Arg Ala Thr Ser
 595 600 605
 5 Val Ala Leu Thr Trp Ser Arg Gly Ser Asp Asn His Ser Pro Ile Ser
 610 615 620
 10 Lys Tyr Thr Ile Gln Thr Lys Thr Ile Leu Ser Asp Asp Trp Lys Asp
 625 630 635 640
 Ala Lys Thr Asp Pro Pro Ile Ile Glu Gly Asn Met Glu Ala Ala Arg
 645 650 655
 15 Ala Val Asp Leu Ile Pro Trp Met Glu Tyr Glu Phe Arg Val Val Ala
 660 665
 Thr Asn Thr Leu Gly Arg Gly Glu Pro Ser Ile Pro Ser Asn Arg Ile
 675 680 685
 20 Lys Thr Asp Gly Ala Ala Pro Asn Val Ala Pro Ser Asp Val Gly Gly
 690 695 700
 25 Gly Gly Gly Arg Asn Arg Glu Leu Thr Ile Thr Trp Ala Pro Leu Ser
 705 710 715 720
 Arg Glu Tyr His Tyr Gly Asn Asn Phe Gly Tyr Ile Val Ala Phe Lys
 725 730 735
 30 Pro Phe Asp Gly Glu Glu Trp Lys Lys Val Thr Val Thr Asn Pro Asp
 740 745 750
 35 Thr Gly Arg Tyr Val His Lys Asp Glu Thr Met Ser Pro Ser Thr Ala
 755 760 765
 40 Phe Gln Val Lys Val Lys Ala Phe Asn Asn Lys Gly Asp Gly Pro Tyr
 770 775 780
 Ser Leu Val Ala Val Ile Asn Ser Ala Gln Asp Ala Pro Ser Glu Ala
 785 790 795 800
 45 Pro Thr Glu Val Gly Val Lys Val Leu Ser Ser Ser Glu Ile Ser Val
 805 810 815
 50 His Trp Glu His Val Leu Glu Lys Ile Val Glu Ser Tyr Gln Ile Arg
 820 825 830
 Tyr Trp Ala Ala His Asp Lys Glu Glu Ala Ala Asn Arg Val Gln Val
 835 840 845
 55

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Thr Ser Gln Glu Tyr Ser Ala Arg Leu Glu Asn Leu Leu Pro Asp Thr
 850 855 860
 5 Gln Tyr Phe Ile Glu Val Gly Ala Cys Asn Ser Ala Gly Cys Gly Pro
 865 870 875 880
 10 Pro Ser Asp Met Ile Glu Ala Phe Thr Lys Lys Ala Pro Pro Ser Gln
 885 890 895
 Pro Pro Arg Ile Ile Ser Ser Val Arg Ser Gly Ser Arg Tyr Ile Ile
 900 905 910
 15 Thr Trp Asp His Val Val Ala Leu Ser Asn Glu Ser Thr Val Thr Gly
 915 920 925
 20 Tyr Lys Val Leu Tyr Arg Pro Asp Gly Gln His Asp Gly Lys Leu Tyr
 930 935 940
 Ser Thr His Lys His Ser Ile Glu Val Pro Ile Pro Arg Asp Gly Glu
 945 950 955 960
 25 Tyr Val Val Glu Val Arg Ala His Ser Asp Gly Gly Asp Gly Val Val
 965 970 975
 30 Ser Gln Val Lys Ile Ser Gly Ala Pro Thr Leu Ser Pro Ser Leu Leu
 980 985 990
 Gly Leu Leu Leu Pro Ala Phe Gly Ile Leu Val Tyr Leu Glu Phe
 995 1000 1005
 35 <210> 5
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 <213> homo sapiens
 40 <400> 5
 Met Lys Thr Gly Leu Phe Phe Leu Cys Leu Leu Gly Thr Ala Ala Ala
 1 5 10 15
 45 Ile Pro Thr Asn Ala Arg Leu Leu Ser Asp His Ser Lys Pro Thr Ala
 20 25 30
 50 Glu Thr Val Ala Pro Asp Asn Thr Ala Ile Pro Ser Leu Arg Ala Glu
 35 40 45
 55 Ala Glu Glu Asn Glu Lys Glu Thr Ala Val Ser Thr Glu Asp Asn Thr
 50 55 60

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

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

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Glu Gly Leu Pro Thr Pro Ile Ile Tyr Trp Ala Lys Glu Asp Gly Met
 290 295 300
 5 Leu Pro Lys Asn Arg Thr Val Tyr Lys Asn Phe Glu Lys Thr Leu Gln
 305 310 315 320
 10 Ile Ile His Val Ser Glu Ala Asp Ser Gly Asn Tyr Gln Cys Ile Ala
 325 330 335
 15 Lys Asn Ala Leu Gly Ala Ile His His Thr Ile Ser Val Arg Val Lys
 340 345 350
 Ala Ala Pro Tyr Trp Ile Thr Ala Pro Gln Asn Leu Val Leu Ser Pro
 355 360 365
 20 Gly Glu Asp Gly Thr Leu Ile Cys Arg Ala Asn Gly Asn Pro Lys Pro
 370 375 380
 25 Arg Ile Ser Trp Leu Thr Asn Gly Val Pro Ile Glu Ile Ala Pro Asp
 385 390 395 400
 Asp Pro Ser Arg Lys Ile Asp Gly Asp Thr Ile Ile Phe Ser Asn Val
 405 410 415
 30 Gln Glu Arg Ser Ser Ala Val Tyr Gln Cys Asn Ala Ser Asn Glu Tyr
 420 425 430
 Gly Tyr Leu Leu Ala Asn Ala Phe Val Asn Val Leu Ala Glu Pro Pro
 435 440 445
 35 Arg Ile Leu Thr Pro Ala Asn Thr Leu Tyr Gln Val Ile Ala Asn Arg
 450 455 460
 40 Pro Ala Leu Leu Asp Cys Ala Phe Phe Gly Ser Pro Leu Pro Thr Ile
 465 470 475 480
 Glu Trp Phe Lys Gly Ala Lys Gly Ser Ala Leu His Glu Asp Ile Tyr
 485 490 495
 45 Val Leu His Glu Asn Gly Thr Leu Glu Ile Pro Val Ala Gln Lys Asp
 500 505 510
 50 Ser Thr Gly Thr Tyr Thr Cys Val Ala Arg Asn Lys Leu Gly Met Ala
 515 520 525
 Lys Asn Glu Val His Leu Glu Ile Lys Asp Ala Thr Trp Ile Val Lys
 530 535 540

55

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5 Gln Pro Glu Tyr Ala Val Val Gln Arg Gly Ser Met Val Ser Phe Glu
 545 550 555 560
 10 Cys Lys Val Lys His Asp His Thr Leu Ser Leu Thr Val Leu Trp Leu
 565 570 575
 15 Lys Asp Asn Arg Glu Leu Pro Ser Asp Glu Arg Phe Thr Val Asp Lys
 580 585 590
 20 Asp His Leu Val Val Ala Asp Val Ser Asp Asp Asp Ser Gly Thr Tyr
 595 600 605
 25 Thr Cys Val Ala Asn Thr Thr Leu Asp Ser Val Ser Ala Ser Ala Val
 610 615 620
 30 Leu Ser Val Val Asp Val Pro Asn Pro Pro Phe Asp Leu Glu Leu Thr
 625 630 635 640
 35 Asp Gln Leu Asp Lys Ser Val Gln Leu Ser Trp Thr Pro Gly Asp Asp
 645 650 655
 40 Asn Asn Ser Pro Ile Thr Lys Phe Ile Ile Glu Tyr Glu Asp Ala Met
 660 665 670
 45 His Lys Pro Gly Leu Trp His His Gln Thr Glu Val Ser Gly Thr Gln
 675 680 685
 50 Thr Thr Ala Gln Leu Lys Leu Ser Pro Tyr Val Asn Tyr Ser Phe Arg
 690 695 700
 55 Val Met Ala Val Asn Ser Ile Gly Lys Ser Leu Pro Ser Glu Ala Ser
 705 710 715 720
 60 Glu Gln Tyr Leu Thr Lys Ala Ser Glu Pro Asp Lys Asn Pro Thr Ala
 725 730 735
 65 Val Glu Gly Leu Gly Ser Glu Pro Asp Asn Leu Val Ile Thr Trp Lys
 740 745 750
 70 Pro Leu Asn Gly Phe Glu Ser Asn Gly Pro Gly Leu Gln Thr Ser Thr
 755 760 765
 75 Ala Ser Phe
 80 770

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<211> 1180

<212> PRT

55 <213> homo sapiens

<400> 7

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

EP 2 462 444 B1

Ala Lys Ser Ser Arg Glu Arg Pro Pro Thr Phe Leu Thr Pro Glu Gly
245 250 255

5 Asn Ala Ser Asn Lys Glu Glu Leu Arg Gly Asn Val Leu Ser Leu Glu
260 265 270

10 Cys Ile Ala Glu Gly Leu Pro Thr Pro Ile Ile Tyr Trp Ala Lys Glu
275 280 285

Asp Gly Met Leu Pro Lys Asn Arg Thr Val Tyr Lys Asn Phe Glu Lys
290 295 300

15 Thr Leu Gln Ile Ile His Val Ser Glu Ala Asp Ser Gly Asn Tyr Gln
305 310 315 320

20 Cys Ile Ala Lys Asn Ala Leu Gly Ala Ile His His Thr Ile Ser Val
325 330 335

Arg Val Lys Ala Ala Pro Tyr Trp Ile Thr Ala Pro Gln Asn Leu Val
340 345 350

25 Leu Ser Pro Gly Glu Asp Gly Thr Leu Ile Cys Arg Ala Asn Gly Asn
355 360 365

30 Pro Lys Pro Arg Ile Ser Trp Leu Thr Asn Gly Val Pro Ile Glu Ile
370 375 380

Ala Pro Asp Asp Pro Ser Arg Lys Ile Asp Gly Asp Thr Ile Ile Phe
385 390 395 400

35 Ser Asn Val Gln Glu Arg Ser Ser Ala Val Tyr Gln Cys Asn Ala Ser
405 410 415

40 Asn Glu Tyr Gly Tyr Leu Leu Ala Asn Ala Phe Val Asn Val Leu Ala
420 425 430

Glu Pro Pro Arg Ile Leu Thr Pro Ala Asn Thr Leu Tyr Gln Val Ile
435 440 445

45 Ala Asn Arg Pro Ala Leu Leu Asp Cys Ala Phe Phe Gly Ser Pro Leu
450 455 460

50 Pro Thr Ile Glu Trp Phe Lys Gly Ala Lys Gly Ser Ala Leu His Glu
465 470 475 480

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

55

EP 2 462 444 B1

					485					490					495	
5	Gln	Lys	Asp	Ser	Thr	Gly	Thr	Tyr	Thr	Cys	Val	Ala	Arg	Asn	Lys	Leu
				500					505					510		
	Gly	Met	Ala	Lys	Asn	Glu	Val	His	Leu	Glu	Ile	Lys	Asp	Ala	Thr	Trp
			515					520					525			
10	Ile	Val	Lys	Gln	Pro	Glu	Tyr	Ala	Val	Val	Gln	Arg	Gly	Ser	Met	Val
		530					535					540				
15	Ser	Phe	Glu	Cys	Lys	Val	Lys	His	Asp	His	Thr	Leu	Ser	Leu	Thr	Val
	545					550					555					560
	Leu	Trp	Leu	Lys	Asp	Asn	Arg	Glu	Leu	Pro	Ser	Asp	Glu	Arg	Phe	Thr
				565						570					575	
20	Val	Asp	Lys	Asp	His	Leu	Val	Val	Ala	Asp	Val	Ser	Asp	Asp	Asp	Ser
				580					585					590		
25	Gly	Thr	Tyr	Thr	Cys	Val	Ala	Asn	Thr	Thr	Leu	Asp	Ser	Val	Ser	Ala
			595					600					605			
	Ser	Ala	Val	Leu	Ser	Val	Val	Ala	Pro	Thr	Pro	Thr	Pro	Ala	Pro	Val
		610					615					620				
30	Tyr	Asp	Val	Pro	Asn	Pro	Pro	Phe	Asp	Leu	Glu	Leu	Thr	Asp	Gln	Leu
	625					630					635					640
	Asp	Lys	Ser	Val	Gln	Leu	Ser	Trp	Thr	Pro	Gly	Asp	Asp	Asn	Asn	Ser
35					645					650					655	
	Pro	Ile	Thr	Lys	Phe	Ile	Ile	Glu	Tyr	Glu	Asp	Ala	Met	His	Lys	Pro
				660					665					670		
40	Gly	Leu	Trp	His	His	Gln	Thr	Glu	Val	Ser	Gly	Thr	Gln	Thr	Thr	Ala
			675					680					685			
	Gln	Leu	Lys	Leu	Ser	Pro	Tyr	Val	Asn	Tyr	Ser	Phe	Arg	Val	Met	Ala
45		690					695					700				
	Val	Asn	Ser	Ile	Gly	Lys	Ser	Leu	Pro	Ser	Glu	Ala	Ser	Glu	Gln	Tyr
	705					710					715					720
50	Leu	Thr	Lys	Ala	Ser	Glu	Pro	Asp	Lys	Asn	Pro	Thr	Ala	Val	Glu	Gly
				725						730					735	
55																

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Leu Gly Ser Glu Pro Asp Asn Leu Val Ile Thr Trp Lys Pro Leu Asn
 740 745 750
 5 Gly Phe Glu Ser Asn Gly Pro Gly Leu Gln Tyr Lys Val Ser Trp Arg
 755 760 765
 10 Gln Lys Asp Gly Asp Asp Glu Trp Thr Ser Val Val Val Ala Asn Val
 770 775 780
 Ser Lys Tyr Ile Val Ser Gly Thr Pro Thr Phe Val Pro Tyr Leu Ile
 785 790 795 800
 15 Lys Val Gln Ala Leu Asn Asp Met Gly Phe Ala Pro Glu Pro Ala Val
 805 810 815
 Val Met Gly His Ser Gly Glu Asp Leu Pro Met Val Ala Pro Gly Asn
 820 825 830
 20 Val Arg Val Asn Val Val Asn Ser Thr Leu Ala Glu Val His Trp Asp
 835 840 845
 25 Pro Val Pro Leu Lys Ser Ile Arg Gly His Leu Gln Gly Tyr Arg Ile
 850 855 860
 Tyr Tyr Trp Lys Thr Gln Ser Ser Ser Lys Arg Asn Arg Arg His Ile
 865 870 875 880
 30 Glu Lys Lys Ile Leu Thr Phe Gln Gly Ser Lys Thr His Gly Met Leu
 885 890 895
 35 Pro Gly Leu Glu Pro Phe Ser His Tyr Thr Leu Asn Val Arg Val Val
 900 905 910
 40 Asn Gly Lys Gly Glu Gly Pro Ala Ser Pro Asp Arg Val Phe Asn Thr
 915 920 925
 Pro Glu Gly Val Pro Ser Ala Pro Ser Ser Leu Lys Ile Val Asn Pro
 930 935 940
 45 Thr Leu Asp Ser Leu Thr Leu Glu Trp Asp Pro Pro Ser His Pro Asn
 945 950 955 960
 50 Gly Ile Leu Thr Glu Tyr Thr Leu Lys Tyr Gln Pro Ile Asn Ser Thr
 965 970 975
 His Glu Leu Gly Pro Leu Val Asp Leu Lys Ile Pro Ala Asn Lys Thr
 980 985 990

55

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Arg Trp Thr Leu Lys Asn Leu Asn Phe Ser Thr Arg Tyr Lys Phe Tyr
 995 1000 1005

5 Phe Tyr Ala Gln Thr Ser Ala Gly Ser Gly Ser Gln Ile Thr Glu
 1010 1015 1020

10 Glu Ala Val Thr Thr Val Asp Glu Ala Met Ala Ser Arg Gln Val
 1025 1030 1035

15 Asp Ile Ala Thr Gln Gly Trp Phe Ile Gly Leu Met Cys Ala Val
 1040 1045 1050

20 Ala Leu Leu Ile Leu Ile Leu Leu Ile Val Cys Phe Ile Arg Arg
 1055 1060 1065

25 Asn Lys Gly Gly Lys Tyr Pro Val Lys Glu Lys Glu Asp Ala His
 1070 1075 1080

30 Ala Asp Pro Glu Ile Gln Pro Met Lys Glu Asp Asp Gly Thr Phe
 1085 1090 1095

35 Gly Glu Tyr Ser Asp Ala Glu Asp His Lys Pro Leu Lys Lys Gly
 1100 1105 1110

40 Ser Arg Thr Pro Ser Asp Arg Thr Val Lys Lys Glu Asp Ser Asp
 1115 1120 1125

45 Asp Ser Leu Val Asp Tyr Gly Glu Gly Val Asn Gly Gln Phe Asn
 1130 1135 1140

50 Glu Asp Gly Ser Phe Ile Gly Gln Tyr Ser Gly Lys Lys Glu Lys
 1145 1150 1155

55 Glu Pro Ala Glu Gly Asn Glu Ser Ser Glu Ala Pro Ser Pro Val
 1160 1165 1170

Asn Ala Met Asn Ser Phe Val
 1175 1180

<210> 8
 <211> 818
 <212> PRT
 <213> homo sapiens

<400> 8

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

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

55

EP 2 462 444 B1

Lys Gln Ala Phe Leu Gln Val Phe Val Gln Pro His Ile Ile Gln Leu
 275 280 285
 5 Lys Asn Glu Thr Thr Tyr Glu Asn Gly Gln Val Thr Leu Val Cys Asp
 290 295 300
 10 Ala Glu Gly Glu Pro Ile Pro Glu Ile Thr Trp Lys Arg Ala Val Asp
 305 310 315 320
 Gly Phe Thr Phe Thr Glu Gly Asp Lys Ser Leu Asp Gly Arg Ile Glu
 325 330 335
 15 Val Lys Gly Gln His Gly Ser Ser Ser Leu His Ile Lys Asp Val Lys
 340 345 350
 20 Leu Ser Asp Ser Gly Arg Tyr Asp Cys Glu Ala Ala Ser Arg Ile Gly
 355 360 365
 Gly His Gln Lys Ser Met Tyr Leu Asp Ile Glu Tyr Ala Pro Lys Phe
 370 375 380
 25 Ile Ser Asn Gln Thr Ile Tyr Tyr Ser Trp Glu Gly Asn Pro Ile Asn
 385 390 395 400
 30 Ile Ser Cys Asp Val Lys Ser Asn Pro Pro Ala Ser Ile His Trp Arg
 405 410 415
 Arg Asp Lys Leu Val Leu Pro Ala Lys Asn Thr Thr Asn Leu Lys Thr
 420 425 430
 35 Tyr Ser Thr Gly Arg Lys Met Ile Leu Glu Ile Ala Pro Thr Ser Asp
 435 440 445
 40 Asn Asp Phe Gly Arg Tyr Asn Cys Thr Ala Thr Asn His Ile Gly Thr
 450 455 460
 Arg Phe Gln Glu Tyr Ile Leu Ala Leu Ala Asp Val Pro Ser Ser Pro
 465 470 475 480
 45 Tyr Gly Val Lys Ile Ile Glu Leu Ser Gln Thr Thr Ala Lys Val Ser
 485 490 495
 50 Phe Asn Lys Pro Asp Ser His Gly Gly Val Pro Ile His His Tyr Gln
 500 505 510
 Val Asp Val Lys Glu Val Ala Ser Glu Ile Trp Lys Ile Val Arg Ser

55

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	515					520					525					
5	His	Gly	Val	Gln	Thr	Met	Val	Val	Leu	Asn	Asn	Leu	Glu	Pro	Asn	Thr
		530					535					540				
	Thr	Tyr	Glu	Ile	Arg	Val	Ala	Ala	Val	Asn	Gly	Lys	Gly	Gln	Gly	Asp
	545					550					555					560
10	Tyr	Ser	Lys	Ile	Glu	Ile	Phe	Gln	Thr	Leu	Pro	Val	Arg	Glu	Pro	Ser
					565					570					575	
15	Pro	Pro	Ser	Ile	His	Gly	Gln	Pro	Ser	Ser	Gly	Lys	Ser	Phe	Lys	Leu
				580					585					590		
	Ser	Ile	Thr	Lys	Gln	Asp	Asp	Gly	Gly	Ala	Pro	Ile	Leu	Glu	Tyr	Ile
			595					600					605			
20	Val	Lys	Tyr	Arg	Ser	Lys	Asp	Lys	Glu	Asp	Gln	Trp	Leu	Glu	Lys	Lys
		610					615					620				
25	Val	Gln	Gly	Asn	Lys	Asp	His	Ile	Ile	Leu	Glu	His	Leu	Gln	Trp	Thr
	625					630					635					640
	Met	Gly	Tyr	Glu	Val	Gln	Ile	Thr	Ala	Ala	Asn	Arg	Leu	Gly	Tyr	Ser
					645					650					655	
30	Glu	Pro	Thr	Val	Tyr	Glu	Phe	Ser	Met	Pro	Pro	Lys	Pro	Asn	Ile	Ile
				660					665					670		
35	Lys	Asp	Thr	Leu	Phe	Asn	Gly	Leu	Gly	Leu	Gly	Ala	Val	Ile	Gly	Leu
			675					680					685			
	Gly	Val	Ala	Ala	Leu	Leu	Leu	Ile	Leu	Val	Val	Thr	Asp	Val	Ser	Cys
		690					695					700				
40	Phe	Phe	Ile	Arg	Gln	Cys	Gly	Leu	Leu	Met	Cys	Ile	Thr	Arg	Arg	Met
	705					710					715					720
45	Cys	Gly	Lys	Lys	Ser	Gly	Ser	Ser	Gly	Lys	Ser	Lys	Glu	Leu	Glu	Glu
					725					730					735	
	Gly	Lys	Ala	Ala	Tyr	Leu	Lys	Asp	Gly	Ser	Lys	Glu	Pro	Ile	Val	Glu
				740					745					750		
50	Met	Arg	Thr	Glu	Asp	Glu	Arg	Val	Thr	Asn	His	Glu	Asp	Gly	Ser	Pro
			755					760					765			
55																

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Val Asn Glu Pro Asn Glu Thr Thr Pro Leu Thr Glu Pro Glu Lys Leu
770 775 780

5 Pro Leu Lys Glu Glu Asp Gly Lys Glu Ala Leu Asn Pro Glu Thr Ile
785 790 795 800

10 Glu Ile Lys Val Ser Asn Asp Ile Ile Gln Ser Lys Glu Asp Asp Ser
805 810 815

Lys Ala

<210> 9
15 <211> 287
<212> PRT
<213> homo sapiens

20 <400> 9

Met Gly Asn Ala Met Phe Val Lys Glu Gln Leu Ser Leu Leu Asp Arg
1 5 10 15

25 Phe Thr Glu Asp Ala Lys Arg Leu Tyr Gly Ser Glu Ala Phe Ala Thr
20 25 30

30 Asp Phe Gln Asp Ser Ala Ala Ala Lys Lys Leu Ile Asn Asp Tyr Val
35 40 45

Lys Asn Gly Thr Arg Gly Lys Ile Thr Asp Leu Ile Lys Asn Leu Asp
50 55 60

35 Ser Gln Thr Met Met Val Leu Val Asn Tyr Ile Phe Phe Lys Ala Lys
65 70 75 80

40 Trp Glu Met Pro Phe Asp Pro Gln Asp Thr His Gln Ser Arg Phe Tyr
85 90 95

Leu Asn Lys Lys Lys Trp Val Met Val Pro Met Met Ser Leu His His
100 105 110

45 Leu Thr Ile Pro Tyr Phe Arg Asp Glu Glu Leu Ser Cys Thr Val Val
115 120 125

50 Glu Leu Lys Tyr Thr Gly Asn Ala Ser Ala Leu Phe Ile Leu Pro Asp
130 135 140

Gln Asp Lys Met Glu Glu Val Glu Ala Met Leu Leu Pro Glu Thr Leu
145 150 155 160

55

EP 2 462 444 B1

Lys Arg Trp Arg Asp Ser Leu Glu Phe Arg Glu Ile Gly Glu Leu Tyr
 165 170 175
 5 Leu Pro Lys Phe Ser Ile Ser Arg Asp Tyr Asn Leu Asn Asp Ile Leu
 180 185 190
 10 Leu Gln Leu Gly Ile Glu Glu Ala Phe Thr Ser Lys Ala Asp Leu Ser
 195 200 205
 Gly Ile Thr Gly Ala Arg Asn Leu Ala Val Ser Gln Val Val His Lys
 210 215 220
 15 Ala Val Leu Asp Val Phe Glu Glu Gly Thr Glu Ala Ser Ala Ala Thr
 225 230 235 240
 20 Ala Val Lys Ile Thr Leu Leu Ser Ala Leu Val Glu Thr Arg Thr Ile
 245 250 255
 Val Arg Phe Asn Arg Pro Phe Leu Met Ile Ile Val Pro Thr Asp Thr
 260 265 270
 25 Gln Asn Ile Phe Phe Met Ser Lys Val Thr Asn Pro Lys Gln Ala
 275 280 285
 <210> 10
 <211> 338
 30 <212> PRT
 <213> homo sapiens
 <400> 10
 35 Met Arg Lys Arg Ala Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser
 1 5 10 15
 40 Leu Arg Ala Thr Ile Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala
 20 25 30
 Gly Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His Asn Glu
 35 40 45
 45 Ser Thr Cys Glu Gln Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp
 50 55 60
 50 Pro Thr Phe Ile Pro Ala Pro Ile Gln Ala Lys Thr Ser Pro Val Asp
 65 70 75 80
 Glu Lys Ala Leu Gln Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp
 85 90 95
 55

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Thr Glu Asp Lys Leu Arg Ala Ala Met Val Gly Met Leu Ala Asn Phe
 100 105 110
 5 Leu Gly Phe Arg Ile Tyr Gly Met His Ser Glu Leu Trp Gly Val Val
 115 120 125
 His Gly Ala Thr Val Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala
 10 130 135 140
 Ser Leu Tyr Leu Gly Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala
 145 150 155 160
 15 Ile Leu Gly Val Pro Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp
 165 170 175
 Ala His Lys Val Leu Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val
 20 180 185 190
 Ala Gln Gly Arg Ala Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val
 195 200 205
 25 Val Gly Val Phe Thr Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val
 210 215 220
 Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp
 30 225 230 235 240
 Phe Thr Glu Leu Asp Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln
 245 250 255
 35 Ala Val Thr Gly Trp Lys Thr Gly Cys Ser Leu Met Gly Ala Ser Val
 260 265 270
 Asp Ser Thr Leu Ala Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met
 40 275 280 285
 Lys Gly Phe Ser Leu Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn
 290 295 300
 45 Ser Thr Ser Val Ser Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln
 305 310 315 320
 His Trp Ser Asp Ile Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe
 50 325 330 335
 Thr Glu

55 <210> 11
 <211> 485
 <212> PRT
 <213> homo sapiens

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<400> 11

5 Met Arg Lys Arg Ala Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser
1 5 10

Leu Arg Ala Thr Ile Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala
20 25 30

10 Gly Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His Asn Glu
35 40 45

15 Ser Thr Cys Glu Gln Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp
50 55 60

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

20 Glu Lys Ala Leu Gln Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp
85 90 95

25 Thr Glu Asp Lys Leu Arg Ala Ala Met Val Gly Met Leu Ala Asn Phe
100 105 110

Leu Gly Phe Arg Ile Tyr Gly Met His Ser Glu Leu Trp Gly Val Val
115 120 125

30 His Gly Ala Thr Val Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala
130 135 140

35 Ser Leu Tyr Leu Gly Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala
145 150 155 160

Ile Leu Gly Val Pro Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp
165 170 175

40 Ala His Lys Val Leu Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val
180 185 190

45 Ala Gln Gly Arg Ala Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val
195 200 205

Val Gly Val Phe Thr Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val
210 215 220

50

55

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Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp
 225 230 235 240
 5 Phe Thr Glu Leu Asp Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln
 245 250 255
 10 Ala Val Thr Gly Trp Lys Thr Gly Cys Ser Leu Met Gly Ala Ser Val
 260 265 270
 Asp Ser Thr Leu Ala Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met
 275 280 285
 15 Lys Gly Phe Ser Leu Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn
 290 295 300
 Ser Thr Ser Val Ser Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln
 305 310 315 320
 20 His Trp Ser Asp Ile Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe
 325 330 335
 25 Thr Glu Ser Ala Cys Leu Leu Leu Ile Gln Pro His Tyr Ala Ser Asp
 340 345 350
 Leu Asp Lys Val Glu Gly Leu Thr Phe Gln Gln Asn Ser Leu Asn Trp
 355 360 365
 30 Met Lys Lys Leu Ser Pro Arg Thr Ile His Leu Thr Met Pro Gln Leu
 370 375 380
 35 Val Leu Gln Gly Ser Tyr Asp Leu Gln Asp Leu Leu Ala Gln Ala Glu
 385 390 395 400
 40 Leu Pro Ala Ile Leu His Thr Glu Leu Asn Leu Gln Lys Leu Ser Asn
 405 410 415
 Asp Arg Ile Arg Val Gly Glu Val Leu Asn Ser Ile Phe Phe Glu Leu
 420 425 430
 45 Glu Ala Asp Glu Arg Glu Pro Thr Glu Ser Thr Gln Gln Leu Asn Lys
 435 440 445
 50 Pro Glu Val Leu Glu Val Thr Leu Asn Arg Pro Phe Leu Phe Ala Val
 450 455 460
 Tyr Asp Gln Ser Ala Thr Ala Leu His Phe Leu Gly Arg Val Ala Asn
 465 470 475 480
 55 Pro Leu Ser Thr Ala
 485

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<210> 12
 <211> 398
 <212> PRT
 <213> homo sapiens

5

<400> 12

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

55

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

5
 Gln Arg Ile Glu Val Leu Tyr Thr Pro Thr Ala Met Ile Arg Pro Asp
 225 230 235 240

10
 Pro Pro His Pro Arg Glu Gly Gln Lys Leu Leu Leu His Cys Glu Gly
 245 250 255

15
 Arg Gly Asn Pro Val Pro Gln Gln Tyr Leu Trp Glu Lys Glu Gly Ser
 260 265 270

20
 Val Pro Pro Leu Lys Met Thr Gln Glu Ser Ala Leu Ile Phe Pro Phe
 275 280 285

25
 Leu Asn Lys Ser Asp Ser Gly Thr Tyr Gly Cys Thr Ala Thr Ser Asn
 290 295 300

30
 Met Gly Ser Tyr Lys Ala Tyr Tyr Thr Leu Asn Val Asn Asp Pro Ser
 305 310 315 320

35
 Pro Val Pro Ser Ser Ser Ser Thr Tyr His Ala Ile Ile Gly Gly Ile
 325 330 335

40
 Val Ala Phe Ile Val Phe Leu Leu Leu Ile Met Leu Ile Phe Leu Gly
 340 345 350

45
 His Tyr Leu Ile Arg His Lys Gly Thr Tyr Leu Thr His Glu Ala Lys
 355 360 365

50
 Gly Ser Asp Asp Ala Pro Asp Ala Asp Thr Ala Ile Ile Asn Ala Glu
 370 375 380

55
 Gly Gly Gln Ser Gly Gly Asp Asp Lys Lys Glu Tyr Phe Ile
 385 390 395

<210> 13
 <211> 350
 <212> PRT
 <213> homo sapiens

<400> 13

Met Gln Arg Leu Gly Ala Thr Leu Leu Cys Leu Leu Leu Ala Ala Ala
 1 5 10 15

Val Pro Thr Ala Pro Ala Pro Ala Pro Thr Ala Thr Ser Ala Pro Val
 20 25 30

EP 2 462 444 B1

Lys Pro Gly Pro Ala Leu Ser Tyr Pro Gln Glu Glu Ala Thr Leu Asn
 35 40 45
 5
 Glu Met Phe Arg Glu Val Glu Glu Leu Met Glu Asp Thr Gln His Lys
 50 55 60
 10
 Leu Arg Ser Ala Val Glu Glu Met Glu Ala Glu Glu Ala Ala Ala Lys
 65 70 75 80
 15
 Ala Ser Ser Glu Val Asn Leu Ala Asn Leu Pro Pro Ser Tyr His Asn
 85 90 95
 20
 Glu Thr Asn Thr Asp Thr Lys Val Gly Asn Asn Thr Ile His Val His
 100 105 110
 25
 Arg Glu Ile His Lys Ile Thr Asn Asn Gln Thr Gly Gln Met Val Phe
 115 120 125
 30
 Ser Glu Thr Val Ile Thr Ser Val Gly Asp Glu Glu Gly Arg Arg Ser
 130 135 140
 35
 His Glu Cys Ile Ile Asp Glu Asp Cys Gly Pro Ser Met Tyr Cys Gln
 145 150 155 160
 40
 Phe Ala Ser Phe Gln Tyr Thr Cys Gln Pro Cys Arg Gly Gln Arg Met
 165 170 175
 45
 Leu Cys Thr Arg Asp Ser Glu Cys Cys Gly Asp Gln Leu Cys Val Trp
 180 185 190
 50
 Gly His Cys Thr Lys Met Ala Thr Arg Gly Ser Asn Gly Thr Ile Cys
 195 200 205
 55
 Asp Asn Gln Arg Asp Cys Gln Pro Gly Leu Cys Cys Ala Phe Gln Arg
 210 215 220
 Gly Leu Leu Phe Pro Val Cys Thr Pro Leu Pro Val Glu Gly Glu Leu
 225 230 235 240
 60
 Cys His Asp Pro Ala Ser Arg Leu Leu Asp Leu Ile Thr Trp Glu Leu
 245 250 255
 65
 Glu Pro Asp Gly Ala Leu Asp Arg Cys Pro Cys Ala Ser Gly Leu Leu
 260 265 270
 70
 Cys Gln Pro His Ser His Ser Leu Val Tyr Val Cys Lys Pro Thr Phe
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5	Val	Gly 290	Ser	Arg	Asp	Gln	Asp 295	Gly	Glu	Ile	Leu	Leu 300	Pro	Arg	Glu	Val
	Pro	Asp	Glu	Tyr	Glu	Val 310	Gly	Ser	Phe	Met	Glu 315	Glu	Val	Arg	Gln	Glu 320
10	Leu	Glu	Asp	Leu	Glu 325	Arg	Ser	Leu	Thr	Glu 330	Glu	Met	Ala	Leu	Arg 335	Glu
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35	Arg	Ser 50	Ser	Met	Ser	Pro	Thr 55	Thr	Asn	Val	Leu	Leu 60	Ser	Pro	Leu	Ser
	Val	Ala	Thr	Ala	Leu	Ser	Ala 70	Leu	Ser	Leu	Gly 75	Ala	Asp	Glu	Arg	Thr 80
40	Glu	Ser	Ile	Ile	His 85	Arg	Ala	Leu	Tyr	Tyr 90	Asp	Leu	Ile	Ser	Ser 95	Pro
45	Asp	Ile	His	Gly 100	Thr	Tyr	Lys	Glu	Leu 105	Leu	Asp	Thr	Val	Thr 110	Ala	Pro
	Gln	Lys	Asn 115	Leu	Lys	Ser	Ala	Ser 120	Arg	Ile	Val	Phe	Glu 125	Lys	Lys	Leu
50	Arg	Ile 130	Lys	Ser	Ser	Phe	Val 135	Ala	Pro	Leu	Glu	Lys 140	Ser	Tyr	Gly	Thr
55	Arg	Pro	Arg	Val	Leu	Thr	Gly	Asn	Pro	Arg	Leu	Asp	Leu	Gln	Glu	Ile

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

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Ala Tyr Gly Glu Lys Lys Ser Arg Leu Ser Asn Leu Ile Lys Leu Ala
 225 230 235 240

5 Gln Lys Val Pro Thr Ala Asp Leu Glu Asp Val Leu Pro Leu Ala Glu
 245 250 255

10 Asp Ile Thr Asn Ile Leu Ser Lys Cys Cys Glu Ser Ala Ser Glu Asp
 260 265 270

15 Cys Met Ala Lys Glu Leu Pro Glu His Thr Val Lys Leu Cys Asp Asn
 275 280 285

20 Leu Ser Thr Lys Asn Ser Lys Phe Glu Asp Cys Cys Gln Glu Lys Thr
 290 295 300

Ala Met Asp Val Phe Val Cys Thr Tyr Phe Met Pro Ala Ala Gln Leu
 305 310 315 320

25 Pro Glu Leu Pro Asp Val Arg Leu Pro Thr Asn Lys Asp Val Cys Asp
 325 330 335

30 Pro Gly Asn Thr Lys Val Met Asp Lys Tyr Thr Phe Glu Leu Ser Arg
 340 345 350

Arg Thr His Leu Pro Glu Val Phe Leu Ser Lys Val Leu Glu Pro Thr
 355 360 365

35 Leu Lys Ser Leu Gly Glu Cys Cys Asp Val Glu Asp Ser Thr Thr Cys
 370 375 380

Phe Asn Ala Lys Gly Pro Leu Leu Lys Lys Glu Leu Ser Ser Phe Ile
 385 390 395 400

40 Asp Lys Gly Gln Glu Leu Cys Ala Asp Tyr Ser Glu Asn Thr Phe Thr
 405 410 415

45 Glu Tyr Lys Lys Lys Leu Ala Glu Arg Leu Lys Ala Lys Leu Pro Glu
 420 425 430

Ala Thr Pro Thr Glu Leu Ala Lys Leu Val Asn Lys Arg Ser Asp Phe
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50 Ala Ser Asn Cys Cys Ser Ile Asn Ser Pro Pro Leu Tyr Cys Asp Ser
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Glu Ile Asp Ala Glu Leu Lys Asn Ile Leu
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 <213> homo sapiens

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

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Gln Asn Leu Ala Leu Arg Asn Thr Gly Met Glu Thr Pro Thr Gly Val
 225 230 235 240
 5 Cys Ala Ala Leu Ala Ala Ala Gly Val Gln Pro His Ser Leu Asp Leu
 245 250 255
 10 Ser His Asn Ser Leu Arg Ala Thr Val Asn Pro Ser Ala Pro Arg Cys
 260 265 270
 Met Trp Ser Ser Ala Leu Asn Ser Leu Asn Leu Ser Phe Ala Gly Leu
 275 280 285
 15 Glu Gln Val Pro Lys Gly Leu Pro Ala Lys Leu Arg Val Leu Asp Leu
 290 295 300
 20 Ser Cys Asn Arg Leu Asn Arg Ala Pro Gln Pro Asp Glu Leu Pro Glu
 305 310 315 320
 Val Asp Asn Leu Thr Leu Asp Gly Asn Pro Phe Leu Val Pro Gly Thr
 325 330 335
 25 Ala Leu Pro His Glu Gly Ser Met Asn Ser Gly Val Val Pro Ala Cys
 340 345 350
 30 Ala Arg Ser Thr Leu Ser Val Gly Val Ser Gly Thr Leu Val Leu Leu
 355 360 365
 Gln Gly Ala Arg Gly Phe Ala
 370 375
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 40 <400> 17
 Met Gly Ala Pro Val Ala Leu Leu Leu Leu Leu Leu Phe Ala Cys Cys
 1 5 10 15
 45 Trp Ala Pro Ser Gly Ala Asn Leu Ser Gln Asp Asp Ser Gln Pro Trp
 20 25 30
 50 Thr Ser Asp Glu Thr Val Val Ala Gly Gly Thr Val Val Leu Lys Cys
 35 40 45
 55 Gln Val Lys Asp His Glu Asp Ser Ser Leu Gln Trp Ser Asn Pro Ala
 50 55 60

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5 Gln Gln Thr Leu Tyr Phe Gly Glu Lys Arg Ala Leu Arg Asp Asn Arg
 65 70 75 80
 Ile Gln Leu Val Thr Ser Thr Pro His Glu Leu Ser Ile Ser Ile Ser
 85 90 95
 10 Asn Val Ala Leu Ala Asp Glu Gly Glu Tyr Thr Cys Ser Ile Phe Thr
 100 105 110
 Met Pro Val Arg Thr Ala Lys Ser Leu Val Thr Val Leu Gly Ile Pro
 115 120 125
 15 Gln Lys Pro Ile Ile Thr Gly Tyr Lys Ser Ser Leu Arg Glu Lys Asp
 130 135 140
 Thr Ala Thr Leu Asn Cys Gln Ser Ser Gly Ser Lys Pro Ala Ala Arg
 145 150 155 160
 20 Leu Thr Trp Arg Lys Gly Asp Gln Glu Leu His Gly Glu Pro Thr Arg
 165 170 175
 25 Ile Gln Glu Asp Pro Asn Gly Lys Thr Phe Thr Val Ser Ser Ser Val
 180 185 190
 30 Thr Phe Gln Val Thr Arg Glu Asp Asp Gly Ala Asn Ile Val Cys Ser
 195 200 205
 Val Asn His Glu Ser Leu Lys Gly Ala Asp Arg Ser Thr Ser Gln Arg
 210 215 220
 35 Ile Glu Val Leu Tyr Thr Pro Thr Ala Met Ile Arg Pro Asp Pro Pro
 225 230 235 240
 40 His Pro Arg Glu Gly Gln Lys Leu Leu Leu His Cys Glu Gly Arg Gly
 245 250 255
 Asn Pro Val Pro Gln Gln Tyr Leu Trp Glu Lys Glu Gly Ser Val Pro
 260 265 270
 45 Pro Leu Lys Met Thr Gln Glu Ser Ala Leu Ile Phe Pro Phe Leu Asn
 275 280 285
 50 Lys Ser Asp Ser Gly Thr Tyr Gly Cys Thr Ala Thr Ser Asn Met Gly
 290 295 300
 Ser Tyr Lys Ala Tyr Tyr Thr Leu Asn Val Asn Asp Pro Ser Pro Val
 305 310 315 320

55

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Pro Ser Ser Ser Ser Thr Tyr His Ala Ile Ile Gly Gly Ile Val Ala
 325 330 335

5 Phe Ile Val Phe Leu Leu Leu Ile Met Leu Ile Phe Leu Gly His Tyr
 340 345 350

10 Leu Ile Arg His Lys Gly Thr Tyr Leu Thr His Glu Ala Lys Gly Ser
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Asp Asp Ala Pro Asp Ala Asp Thr Ala Ile Ile Asn Ala Glu Gly Gly
 370 375 380

15 Gln Ser Gly Gly Asp Asp Lys Lys Glu Tyr Phe Ile
 385 390 395

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 20 <212> PRT
 <213> homo sapiens

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25 Met Gly Ala Pro Ala Ala Ser Leu Leu Leu Leu Leu Leu Phe Ala
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30 Cys Cys Trp Ala Pro Gly Gly Ala Asn Leu Ser Gln Asp Asp Ser Gln
 20 25 30

Pro Trp Thr Ser Asp Glu Thr Val Val Ala Gly Gly Thr Val Val Leu
 35 40 45

Lys Cys Gln Val Lys Asp His Glu Asp Ser Ser Leu Gln Trp Ser Asn
 50 55 60

40 Pro Ala Gln Gln Thr Leu Tyr Phe Gly Glu Lys Arg Ala Leu Arg Asp
 65 70 75 80

Asn Arg Ile Gln Leu Val Thr Ser Thr Pro His Glu Leu Ser Ile Ser
 85 90 95

45 Ile Ser Asn Val Ala Leu Ala Asp Glu Gly Glu Tyr Thr Cys Ser Ile
 100 105 110

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

Ile Pro Gln Lys Pro Ile Ile Thr Gly Tyr Lys Ser Ser Leu Arg Glu
 130 135 140

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Lys Asp Thr Ala Thr Leu Asn Cys Gln Ser Ser Gly Ser Lys Pro Ala
 145 150 155 160
 5 Ala Arg Leu Thr Trp Arg Lys Gly Asp Gln Glu Leu His Gly Glu Pro
 165 170 175
 Thr Arg Ile Gln Glu Asp Pro Asn Gly Lys Thr Phe Thr Val Ser Ser
 180 185 190
 10 Ser Val Thr Phe Gln Val Thr Arg Glu Asp Asp Gly Ala Ser Ile Val
 195 200 205
 Cys Ser Val Asn His Glu Ser Leu Lys Gly Ala Asp Arg Ser Thr Ser
 210 215 220
 15 Gln Arg Ile Glu Val Leu Tyr Thr Pro Thr Ala Met Ile Arg Pro Asp
 225 230 235 240
 20 Pro Pro His Pro Arg Glu Gly Gln Lys Leu Leu Leu His Cys Glu Gly
 245 250 255
 Arg Gly Asn Pro Val Pro Gln Gln Tyr Leu Trp Glu Lys Glu Gly Ser
 260 265 270
 25 Val Pro Pro Leu Lys Met Thr Gln Glu Ser Ala Leu Ile Phe Pro Phe
 275 280 285
 30 Leu Asn Lys Ser Asp Ser Gly Thr Tyr Gly Cys Thr Ala Thr Ser Asn
 290 295 300
 Met Gly Ser Tyr Lys Ala Tyr Tyr Thr Leu Asn Val Asn Asp Pro Ser
 305 310 315 320
 35 Pro Val Pro Ser Ser Ser Ser Thr Tyr His Ala Ile Ile Gly Gly Ile
 325 330 335
 40 Val Ala Phe Ile Val Phe Leu Leu Leu Ile Met Leu Ile Phe Leu Gly
 340 345 350
 His Tyr Leu Ile Arg His Lys Gly Thr Tyr Leu Thr His Glu Ala Lys
 355 360 365
 45 Gly Ser Asp Asp Ala Pro Asp Ala Asp Thr Ala Ile Ile Asn Ala Glu
 370 375 380
 50 Gly Gly Gln Ser Gly Gly Asp Asp Lys Lys Glu Tyr Phe Ile
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EP 2 462 444 B1

<212> PRT

<213> homo sapiens

<400> 19

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 Arg Ala Met Glu Pro Leu Leu Leu Gly Arg Gly Leu Ile Val Tyr Leu
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 Met Phe Leu Leu Leu Lys Phe Ser Lys Ala Ile Glu Ile Pro Ser Ser
 20 25 30

15
 Val Gln Gln Val Pro Thr Ile Ile Lys Gln Ser Lys Val Gln Val Ala
 35 40 45

20
 Phe Pro Phe Asp Glu Tyr Phe Gln Ile Glu Cys Glu Ala Lys Gly Asn
 50 55 60

25
 Pro Glu Pro Thr Phe Ser Trp Thr Lys Asp Gly Asn Pro Phe Tyr Phe
 65 70 75 80

25
 Thr Asp His Arg Ile Ile Pro Ser Asn Asn Ser Gly Thr Phe Arg Ile
 85 90 95

30
 Pro Asn Glu Gly His Ile Ser His Phe Gln Gly Lys Tyr Arg Cys Phe
 100 105 110

35
 Ala Ser Asn Lys Leu Gly Ile Ala Met Ser Glu Glu Ile Glu Phe Ile
 115 120 125

40
 Val Pro Ser Val Pro Lys Phe Pro Lys Glu Lys Ile Asp Pro Leu Glu
 130 135 140

45
 Val Glu Glu Gly Asp Pro Ile Val Leu Pro Cys Asn Pro Pro Lys Gly
 145 150 155 160

50
 Leu Pro Pro Leu His Ile Tyr Trp Met Asn Ile Glu Leu Glu His Ile
 165 170 175

55
 Glu Gln Asp Glu Arg Val Tyr Met Ser Gln Lys Gly Asp Leu Tyr Phe
 180 185 190

60
 Ala Asn Val Glu Glu Lys Asp Ser Arg Asn Asp Tyr Cys Cys Phe Ala
 195 200 205

65
 Ala Phe Pro Arg Leu Arg Thr Ile Val Gln Lys Met Pro Met Lys Leu

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5	Thr 225	Val	Asn	Ser	Ser	Asn 230	Ser	Ile	Lys	Gln	Arg 235	Lys	Pro	Lys	Leu	Leu 240
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10	Gly	Glu	Ile	Leu 260	Leu	Leu	Glu	Cys	Phe 265	Ala	Glu	Gly	Leu	Pro 270	Thr	Pro
15	Gln	Val	Asp 275	Trp	Asn	Lys	Ile	Gly 280	Gly	Asp	Leu	Pro	Lys 285	Gly	Arg	Glu
	Ala	Lys 290	Glu	Asn	Tyr	Gly	Lys 295	Thr	Leu	Lys	Ile	Glu 300	Asn	Val	Ser	Tyr
20	Gln 305	Asp	Lys	Gly	Asn	Tyr 310	Arg	Cys	Thr	Ala	Ser 315	Asn	Phe	Leu	Gly	Thr 320
25	Ala	Thr	His	Asp	Phe 325	His	Val	Ile	Val	Glu 330	Glu	Pro	Pro	Arg	Trp 335	Thr
	Lys	Lys	Pro	Gln 340	Ser	Ala	Val	Tyr	Ser 345	Thr	Gly	Ser	Asn	Gly 350	Ile	Leu
30	Leu	Cys	Glu 355	Ala	Glu	Gly	Glu	Pro 360	Gln	Pro	Thr	Ile	Lys 365	Trp	Arg	Val
35	Asn	Gly 370	Ser	Pro	Val	Asp	Asn 375	His	Pro	Phe	Ala	Gly 380	Asp	Val	Val	Phe
	Pro 385	Arg	Glu	Ile	Ser	Phe 390	Thr	Asn	Leu	Gln	Pro 395	Asn	His	Thr	Ala	Val 400
40	Tyr	Gln	Cys	Glu	Ala 405	Ser	Asn	Val	His	Gly 410	Thr	Ile	Leu	Ala	Asn 415	Ala
45	Asn	Ile	Asp	Val 420	Val	Asp	Val	Arg	Pro 425	Leu	Ile	Gln	Thr	Lys 430	Asp	Gly
	Glu	Asn	Tyr 435	Ala	Thr	Val	Val	Gly 440	Tyr	Ser	Ala	Phe	Leu 445	His	Cys	Glu
50	Phe	Phe	Ala	Ser	Pro	Glu	Ala 455	Val	Val	Ser	Trp	Gln 460	Lys	Val	Glu	Glu

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Val Lys Pro Leu Glu Gly Arg Arg Tyr His Ile Tyr Glu Asn Gly Thr
 465 470 475 480
 5 Leu Gln Ile Asn Arg Thr Thr Glu Glu Asp Ala Gly Ser Tyr Ser Cys
 485 490 495
 10 Trp Val Glu Asn Ala Ile Gly Lys Thr Ala Val Thr Ala Asn Leu Asp
 500 505 510 515
 Ile Arg Asn Ala Thr Lys Leu Arg Val Ser Pro Lys Asn Pro Arg Ile
 515 520 525
 15 Pro Lys Leu His Met Leu Glu Leu His Cys Glu Ser Lys Cys Asp Ser
 530 535 540
 20 His Leu Lys His Ser Leu Lys Leu Ser Trp Ser Lys Asp Gly Glu Ala
 545 550 555 560
 Phe Glu Ile Asn Gly Thr Glu Asp Gly Arg Ile Ile Ile Asp Gly Ala
 565 570 575
 25 Asn Leu Thr Ile Ser Asn Val Thr Leu Glu Asp Gln Gly Ile Tyr Cys
 580 585 590
 30 Cys Ser Ala His Thr Ala Leu Asp Ser Ala Ala Asp Ile Thr Gln Val
 595 600 605
 Thr Val Leu Asp Val Pro Asp Pro Pro Glu Asn Leu His Leu Ser Glu
 610 615 620
 35 Arg Gln Asn Arg Ser Val Arg Leu Thr Trp Glu Ala Gly Ala Asp His
 625 630 635 640
 40 Asn Ser Asn Ile Ser Glu Tyr Ile Val Glu Phe Glu Gly Asn Lys Glu
 645 650 655
 Glu Pro Gly Arg Trp Glu Glu Leu Thr Arg Val Gln Gly Lys Lys Thr
 660 665 670
 45 Thr Val Ile Leu Pro Leu Ala Pro Phe Val Arg Tyr Gln Phe Arg Val
 675 680 685
 50 Ile Ala Val Asn Glu Val Gly Arg Ser Gln Pro Ser Gln Pro Ser Asp
 690 695 700
 55 His His Glu Thr Pro Pro Ala Ala Pro Asp Arg Asn Pro Gln Asn Ile
 705 710 715 720

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Arg Val Gln Ala Ser Gln Pro Lys Glu Met Ile Ile Lys Trp Glu Pro
 725 730 735
 5 Leu Lys Ser Met Glu Gln Asn Gly Pro Gly Leu Glu Tyr Arg Val Thr
 740 745 750
 10 Trp Lys Pro Gln Gly Ala Pro Val Glu Trp Glu Glu Glu Thr Val Thr
 755 760 765
 15 Asn His Thr Leu Arg Val Met Thr Pro Ala Val Tyr Ala Pro Tyr Asp
 770 775 780
 20 Val Lys Val Gln Ala Ile Asn Gln Leu Gly Ser Gly Pro Asp Pro Gln
 785 790 800
 25 Ser Val Thr Leu Tyr Ser Gly Glu Asp Tyr Pro Asp Thr Ala Pro Val
 805 810 815
 30 Ile His Gly Val Asp Val Ile Asn Ser Thr Leu Val Lys Val Thr Trp
 820 825 830
 35 Ser Thr Val Pro Lys Asp Arg Val His Gly Arg Leu Lys Gly Tyr Gln
 835 840 845
 40 Ile Asn Trp Trp Lys Thr Lys Ser Leu Leu Asp Gly Arg Thr His Pro
 850 855 860
 45 Lys Glu Val Asn Ile Leu Arg Phe Ser Gly Gln Arg Asn Ser Gly Met
 865 870 875 880
 50 Val Pro Ser Leu Asp Ala Phe Ser Glu Phe His Leu Thr Val Leu Ala
 885 890 895
 55 Tyr Asn Ser Lys Gly Ala Gly Pro Glu Ser Glu Pro Tyr Ile Phe Gln
 900 905 910
 Thr Pro Glu Gly Val Pro Glu Gln Pro Thr Phe Leu Lys Val Ile Lys
 915 920 925
 60 Val Asp Lys Asp Thr Ala Thr Leu Ser Trp Gly Leu Pro Lys Lys Leu
 930 935 940
 65 Asn Gly Asn Leu Thr Gly Tyr Leu Leu Gln Tyr Gln Ile Ile Asn Asp
 945 950 955 960
 70 Thr Tyr Glu Ile Gly Glu Leu Asn Asp Ile Asn Ile Thr Thr Pro Ser
 965 970 975
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Lys Pro Ser Trp His Leu Ser Asn Leu Asn Ala Thr Thr Lys Tyr Lys
 980 985 990

5 Phe Tyr Leu Arg Ala Cys Thr Ser Gln Gly Cys Gly Lys Pro Ile Thr
 995 1000 1005

10 Glu Glu Ser Ser Thr Leu Gly Glu Gly Ser Lys Gly Ile Gly Lys
 1010 1015 1020

Ile Ser Gly Val Asn Leu Thr Gln Lys Thr His Pro Val Glu Val
 1025 1030 1035

15 Phe Glu Pro Gly Ala Glu His Ile Val Arg Leu Met Thr Lys Asn
 1040 1045 1050

20 Trp Gly Asp Asn Asp Ser Ile Phe Gln Asp Val Ile Glu Thr Arg
 1055 1060 1065

Gly Arg Glu Tyr Ala Gly Leu Tyr Asp Asp Ile Ser Thr Gln Gly
 1070 1075 1080

25 Trp Phe Ile Gly Leu Met Cys Ala Ile Ala Leu Leu Thr Leu Leu
 1085 1090 1095

30 Leu Leu Thr Val Cys Phe Val Lys Arg Asn Arg Gly Gly Lys Tyr
 1100 1105 1110

Ser Val Lys Glu Lys Glu Asp Leu His Pro Asp Pro Glu Ile Gln
 1115 1120 1125

35 Ser Val Lys Asp Glu Thr Phe Gly Glu Tyr Ser Asp Ser Asp Glu
 1130 1135 1140

40 Lys Pro Leu Lys Gly Ser Leu Arg Ser Leu Asn Arg Asp Met Gln
 1145 1150 1155

Pro Thr Glu Ser Ala Asp Ser Leu Val Glu Tyr Gly Glu Gly Asp
 1160 1165 1170

45 His Gly Leu Phe Ser Glu Asp Gly Ser Phe Ile Gly Ala Tyr Ala
 1175 1180 1185

50 Gly Ser Lys Glu Lys Gly Ser Val Glu Ser Asn Gly Ser Ser Thr
 1190 1195 1200

Ala Thr Phe Pro Leu Arg Ala

55 1205 1210

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<211> 449
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Met Met Lys Thr Leu Leu Leu Phe Val Gly Leu Leu Leu Thr Trp Glu
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Ser Gly Gln Val Leu Gly Asp Gln Thr Val Ser Asp Asn Glu Leu Gln
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Glu Met Ser Asn Gln Gly Ser Lys Tyr Val Asn Lys Glu Ile Gln Asn
 35 40 45

Ala Val Asn Gly Val Lys Gln Ile Lys Thr Leu Ile Glu Lys Thr Asn
 50 55 60

Glu Glu Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu Ala Lys Lys Lys
 65 70 75 80

Lys Glu Asp Ala Leu Asn Glu Thr Arg Glu Ser Glu Thr Lys Leu Lys
 85 90 95

Glu Leu Pro Gly Val Cys Asn Glu Thr Met Met Ala Leu Trp Glu Glu
 100 105 110

Cys Lys Pro Cys Leu Lys Gln Thr Cys Met Lys Phe Tyr Ala Arg Val
 115 120 125

Cys Arg Ser Gly Ser Gly Leu Val Gly Arg Gln Leu Glu Glu Phe Leu
 130 135 140

Asn Gln Ser Ser Pro Phe Tyr Phe Trp Met Asn Gly Asp Arg Ile Asp
 145 150 155 160

Ser Leu Leu Glu Asn Asp Arg Gln Gln Thr His Met Leu Asp Val Met
 165 170 175

Gln Asp His Phe Ser Arg Ala Ser Ser Ile Ile Asp Glu Leu Phe Gln
 180 185 190

Asp Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu Pro
 195 200 205

Phe Ser Leu Pro His Arg Arg Pro His Phe Phe Phe Pro Lys Ser Leu

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Met Gln Asp His Phe Ser Arg Ala Ser Ser Ile Ile Asp Glu Leu Phe
 1 5 10

5 Gln Asp Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu
 20 25 30

Pro Phe Ser Leu Pro His Arg Arg Pro His Phe Phe Phe Pro Lys Ser
 35 40 45

10 Arg Ile Val Arg Ser Leu Met Pro Phe Ser Pro Tyr Glu Pro Leu Asn
 50 55 60

15 Phe His Ala Met Phe Gln Pro Phe Leu Glu Met Ile His Glu Ala Gln
 65 70 75 80

20 Gln Ala Met Asp Ile His Phe His Ser Pro Ala Phe Gln His Pro Pro
 85 90 95

Thr Glu Phe Ile Arg Glu Gly Asp Asp Asp Arg Thr Val Cys Arg Glu
 100 105 110

25 Ile Arg His Asn Ser Thr Gly Cys Leu Arg Met Lys Asp Gln Cys Asp
 115 120 125

30 Lys Cys Arg Glu Ile Leu Ser Val Asp Cys Ser Thr Asn Asn Pro Ser
 130 135 140

Gln Ala Lys Leu Arg Arg Glu Leu Asp Glu Ser Leu Gln Val Ala Glu
 145 150 155 160

35 Arg Leu Thr Arg Lys Tyr Asn Glu Leu Leu Lys Ser Tyr Gln Trp Lys
 165 170 175

40 Met Leu Asn Thr Ser Ser Leu Leu Glu Gln Leu Asn Glu Gln Phe Asn
 180 185 190

Trp Val Ser Arg Leu Ala Asn Leu Thr Gln Gly Glu Asp Gln Tyr Tyr
 195 200 205

45 Leu Arg Val Thr Thr Val Ala Ser His Thr Ser Asp Ser Asp Val Pro
 210 215 220

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55

EP 2 462 444 B1

Ser Gly Val Thr Glu Val Val Val Lys Leu Phe Asp Ser Asp Pro Ile
 225 230 235 240
 5 Thr Val Thr Val Pro Val Glu Val Ser Arg Lys Asn Pro Lys Phe Met
 245 250 255
 10 Glu Thr Val Ala Glu Lys Ala Leu Gln Glu Tyr Arg Lys Lys His Arg
 260 265 270
 Glu Glu
 <210> 22
 15 <211> 253
 <212> PRT
 <213> homo sapiens
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 20 Gly Ser Ser Glu His Leu Lys Arg Glu His Ser Leu Ile Lys Pro Tyr
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 25 Gln Gly Val Gly Ser Ser Ser Met Pro Leu Trp Asp Phe Gln Gly Ser
 20 25 30
 30 Thr Ile Leu Thr Ser Gln Tyr Val Arg Leu Thr Pro Asp Glu Arg Ser
 35 40 45
 35 Lys Glu Gly Ser Ile Trp Asn His Gln Pro Cys Phe Leu Lys Asp Trp
 50 55 60
 40 Glu Met His Val His Phe Lys Val His Gly Thr Gly Lys Lys Asn Leu
 65 70 75 80
 45 His Gly Asp Gly Ile Ala Leu Trp Tyr Thr Arg Asp Arg Leu Val Pro
 85 90 95
 50 Gly Pro Val Phe Gly Ser Lys Asp Asn Phe His Gly Leu Ala Ile Phe
 100 105 110
 55 Leu Asp Thr Tyr Pro Asn Asp Glu Thr Thr Glu Arg Val Phe Pro Tyr
 115 120 125
 50 Ile Ser Val Met Val Asn Asn Gly Ser Leu Ser Tyr Asp His Ser Lys
 130 135 140
 Asp Gly Arg Trp Thr Glu Leu Ala Gly Cys Thr Ala Asp Phe Arg Asn
 145 150 155 160
 55

EP 2 462 444 B1

Arg Asp His Asp Thr Phe Leu Ala Val Arg Tyr Ser Arg Gly Arg Leu
 165 170 175
 5 Thr Val Met Thr Asp Leu Glu Asp Lys Asn Glu Trp Lys Asn Cys Ile
 180 185 190
 10 Asp Ile Thr Gly Val Arg Leu Pro Thr Gly Tyr Tyr Phe Gly Ala Ser
 195 200 205
 15 Ala Gly Thr Gly Asp Leu Ser Asp Asn His Asp Ile Ile Ser Met Lys
 210 215 220
 20 Leu Phe Gln Leu Met Val Glu His Thr Pro Asp Glu Glu Asn Ile Asp
 225 230 235 240
 Trp Thr Lys Ile Glu Pro Ser Val Asn Phe Leu Lys Ser
 245 250
 <210> 23
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 25 <400> 23
 30 Met Gln Val Cys Ser Gln Pro Gln Arg Gly Cys Val Arg Glu Gln Ser
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 20 25 30
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 45 Arg Ile Gly Gly Met Met Lys Thr Leu Leu Leu Phe Val Gly Leu Leu
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 50 Leu Thr Trp Glu Ser Gly Gln Val Leu Gly Asp Gln Thr Val Ser Asp
 65 70 75 80
 55 Asn Glu Leu Gln Glu Met Ser Asn Gln Gly Ser Lys Tyr Val Asn Lys
 85 90 95
 60 Glu Ile Gln Asn Ala Val Asn Gly Val Lys Gln Ile Lys Thr Leu Ile
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 65 Glu Lys Thr Asn Glu Glu Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu
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 70 75

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

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Val Ala Glu Arg Leu Thr Arg Lys Tyr Asn Glu Leu Leu Lys Ser Tyr
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 55

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Phe Ala Leu Glu Gly Phe Pro Thr Glu Pro Asn Ser Ser Ser Arg Ala
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 5 Ile His Val His Gln Phe Gly Asp Leu Ser Gln Gly Cys Glu Ser Thr
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 15 Ala Gly Leu Ala Ala Ser Leu Ala Gly Pro His Ser Ile Val Gly Arg
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 55

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 50 55 60
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 50 Pro Lys Ile Val Leu Gly Gln Glu Gln Asp Ser Tyr Gly Gly Lys Phe
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 55

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35 40 45

30 His Ser Thr Lys Pro Pro Arg Tyr Lys Cys Gly Ile Ser Lys Ala Cys
50 55 60

Pro Glu Lys His Phe Ala Phe Lys Met Ala Ser Gly Ala Ala Asn Val
65 70 75 80

35 Val Gly Pro Lys Ile Cys Leu Glu Asp Asn Val Leu Met Ser Gly Val
85 90 95

40 Lys Asn Asn Val Gly Arg Gly Ile Asn Val Ala Leu Ala Asn Gly Lys
100 105 110

Thr Gly Glu Val Leu Asp Thr Lys Tyr Phe Asp Met Trp Gly Gly Asp
115 120 125

45 Val Ala Pro Phe Ile Glu Phe Leu Lys Ala Ile Gln Asp Gly Thr Ile
130 135 140

50 Val Leu Met Gly Thr Tyr Asp Asp Gly Ala Thr Lys Leu Asn Asp Glu
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Ala Arg Arg Leu Ile Ala Asp Leu Gly Ser Thr Ser Ile Thr Asn Leu
165 170 175

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Gly Phe Arg Asp Asn Trp Val Phe Cys Gly Gly Lys Gly Ile Lys Thr
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 5 Lys Ser Pro Phe Glu Gln His Ile Lys Asn Asn Lys Asp Thr Asn Lys
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 65 70 75 80
 40 Asn Arg Ile Gln Leu Val Thr Ser Thr Pro His Glu Leu Ser Ile Ser
 85 90 95
 45 Ile Ser Asn Val Ala Leu Ala Asp Glu Gly Glu Tyr Thr Cys Ser Ile
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 Phe Thr Met Pro Val Arg Thr Ala Lys Ser Leu Val Thr Val Leu Gly
 115 120 125
 50 Ile Pro Gln Lys Pro Ile Ile Thr Gly Tyr Lys Ser Ser Leu Arg Glu
 130 135 140
 Lys Asp Thr Ala Thr Leu Asn Cys Gln Ser Ser Gly Ser Lys Pro Ala
 145 150 155 160
 55

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Ala Arg Leu Thr Trp Arg Lys Gly Asp Gln Glu Leu His Gly Glu Pro
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5 Thr Arg Ile Gln Glu Asp Pro Asn Gly Lys Thr Phe Thr Val Ser Ser
180 185 190

10 Ser Val Thr Phe Gln Val Thr Arg Glu Asp Asp Gly Ala Ser Ile Val
195 200 205

15 Cys Ser Val Asn His Glu Ser Leu Lys Gly Ala Asp Arg Ser Thr Ser
210 215 220

Gln Arg Ile Glu Val Leu Tyr Thr Pro Thr Ala Met Ile Arg Pro Asp
225 230 235 240

20 Pro Pro His Pro Arg Glu Gly Gln Lys Leu Leu Leu His Cys Glu Gly
245 250 255

Arg Gly Asn Pro Val Pro Gln Gln Tyr Leu Trp Glu Lys Glu Gly Ser
260 265 270

25 Val Pro Pro Leu Lys Met Thr Gln Glu Ser Ala Leu Ile Phe Pro Phe
275 280 285

30 Leu Asn Lys Ser Asp Ser Gly Thr Tyr Gly Cys Thr Ala Thr Ser Asn
290 295 300

Met Gly Ser Tyr Lys Ala Tyr Tyr Thr Leu Asn Val Asn Asp Pro Ser
305 310 315 320

35 Pro Val Pro Ser Ser Ser Ser Thr Tyr His Ala Ile Ile Gly Gly Ile
325 330 335

40 Val Ala Phe Ile Val Phe Leu Leu Leu Ile Met Leu Ile Phe Leu Gly
340 345 350

His Tyr Leu Ile Arg His Lys Gly Thr Tyr Leu Thr His Glu Ala Lys
355 360 365

45 Gly Ser Asp Asp Ala Pro Asp Ala Asp Thr Ala Ile Ile Asn Ala Glu
370 375 380

50 Gly Gly Gln Ser Gly Gly Asp Asp Lys Lys Glu Tyr Phe Ile
385 390 395

Claims

- 55 1. Use of a device in an in vitro method for detecting the presence or absence of cerebrospinal fluid (CSF) in a sample, said method comprising contacting the sample with a binding partner specific for a CSF-enriched protein, and detecting binding partner-CSF-enriched protein complexes if present, wherein the presence of detectable complexes

indicates the presence of said CSF-enriched protein in the sample;
 wherein the CSF-enriched protein is cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched protein,

5 said device comprising
 a sample application region,
 a sample labeling region comprising a first antibody to said CSF-enriched protein, wherein the first antibody is conjugated to a mobile particle;
 a sample detection region comprising a second antibody to said CSF-enriched protein, wherein the second antibody
 10 is fixed to the sample detection region,
 wherein the formation of a detectable band in the second region following application of the sample to the sample application region indicates the presence of said CSF-enriched protein in the sample.

15 2. The use of claim 1, wherein the device comprises two to ten different antibodies that each specifically binds a different CSF-enriched protein.

3. The use of claim 2, wherein the device provides a single combined result, or the device provides an individual result for each antibody, or
 20 wherein each antibody is employed at a subthreshold level.

4. The use of claim 2, wherein the device comprises four to ten different antibodies that each specifically binds a different CSF-enriched protein, and wherein a positive test does not require binding to all antibodies.

5. The use of any one of claims 1-4, wherein the first antibody to said CSF-enriched protein binds a post-translational
 25 modification in the CSF-enriched protein.

6. The use of any one of claims 2-5, wherein the two to ten antibodies are selected from

30 (i) an antibody that binds cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof; and

(ii) an antibody that binds CNTN2 Contactin-2 precursor (SEQ ID NO: 3; Accession Number gi|4827022); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (SEQ ID NO: 6; Accession Number: gi|68534652 and SEQ ID NO: 7; Accession Number: gi|109731501); Isoform 1 of Neural cell adhesion molecule-like (SEQ ID NO: 1; Accession Number gi:62088238) protein; Chain A,
 35 Human Mesotrypsin Complexed With Bovine Pancreatic Trypsin Inhibitor(Bpti) (SEQ ID NO:2; Accession number gi:162330095); CNTN1 Isoform 2 of Contactin-1 (SEQ ID NO: 4; Accession Number gi:28373119); cDNA highly similar to SPARC-like protein 1 (unnamed protein product) (SEQ ID NO: 5; Accession Number: gi|194388050); NCAM2 Neural cell adhesion molecule 2, isoform CRA_a (SEQ ID NO: 8; Accession Number gi|119630409); SERPINA3 serpin peptidase inhibitor, clade A, member 3 precursor /Isoform 1 of Alpha-1-
 40 antichymotrypsin/growth-inhibiting protein 25 [Homo sapiens] or slightly longer fragment of alpha-1-antichymotrypsin precursor (SEQ ID NO: 9; Accession Number gi|46981961); AGT Angiotensinogen (SEQ ID NO: 10; Accession Number gi|553181); Angiotensinogen precursor (Serpin A8) (SEQ ID NO: 11; Accession Number gi|4557287); unnamed protein product also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 12; Accession Number gi|187608363); SERPINF1 serine (or
 45 cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor, Pedf), member 1 isoform 4 factor (SEQ ID NO: 14; Accession Number gi|15988024); human protein similar to GC Vitamin D-binding protein PREDICTED: vitamin D-binding protein [Pan troglodytes] (SEQ ID NO: 15; Accession Number 181482); CD14 Human monocyte antigen CD14 (CD14) (SEQ ID NO: 16; Accession Number gi|117646212); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (SEQ ID NO: 17; Accession
 50 Number gi|90080503; SEQ ID NO: 18; gi|187608363 (human); Neural cell adhesion molecule variant (SEQ ID NO: 19; Accession Number gi:62088238); unnamed protein similar to CLU cDNA FLJ57622, highly similar to Clusterin (SEQ ID NO: 20; Accession number gi|189054091); protein highly similar to Clusterin (SEQ ID NO: 21; Accession number gi|193787502); LMAN2 Vesicular integral-membrane protein VIP36 (SEQ ID NO: 22; Accession number gi|157834800); clusterin isoform 1 [Homo sapiens] (SEQ ID NO: 23; Accession number
 55 NM_001831.2); superoxide dismutase 3, extracellular precursor (SEQ ID NO: 24; Accession number gi|118582275); fibrin alpha C term fragment (SEQ ID NO: 25; Accession number gi|223057); Chain A, Human Kallikrein 6 (Hk6) Active Form or KLK6 Isoform 1 of Kallikrein-6 (SEQ ID NO: 26; Accession number gi|21465970); APCS Serum amyloid P-component /Chain A or Pentameric Human Serum Amyloid P Component (SEQ ID

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NO: 27; Accession number gi|576259); FAM3C Protein FAM3C / family with sequence similarity 3, member C precursor [Homo sapiens] note="predicted osteoblast protein; interleukin-like EMT inducer (SEQ ID NO: 28; Accession number gi|55629272); protein similar to unnamed protein product [Macaca fascicularis] also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 29; Accession number gi|187608363); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched proteins.

7. The use of any one of claims 2-5, wherein the two to ten different antibodies are selected from

(i) an antibody that binds cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form thereof; and
(ii) an antibody that binds CNTN2 Contactin-2 precursor (SEQ ID NO: 3; Accession Number gi|4827022); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (SEQ ID NO: 6; Accession Number: gi|68534652 and SEQ ID NO: 7); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (SEQ ID NO: 17); Neural cell adhesion molecule variant (SEQ ID NO: 19; Accession Number gi:62088238); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched proteins.

8. An *in vitro* method for detecting the presence or absence of CSF in a sample, said method comprising contacting the sample with a binding partner specific for a CSF-enriched protein, and detecting binding partner-CSF-enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of said CSF-enriched protein in the sample; wherein the CSF-enriched protein is cDNA FLJ59893, dickkopf homolog 3 precursor (SEQ ID NO: 13; Accession Number gi|40548389); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched protein.

9. The method of claim 8, further comprising contacting the sample with a binding partner specific for a second CSF-enriched protein, and detecting the binding partner-second CSF-enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of said second CSF-enriched protein in the sample; wherein the second CSF-enriched protein is CNTN2 Contactin-2 precursor (SEQ ID NO: 3; Accession Number gi|4827022); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (SEQ ID NO: 6; Accession Number: gi|68534652 and SEQ ID NO: 7; Accession Number: gi|109731501); Isoform 1 of Neural cell adhesion molecule-like (SEQ ID NO: 1; Accession Number gi:62088238) protein; Chain A, Human Mesotrypsin Complexed With Bovine Pancreatic Trypsin Inhibitor(Bpti) (SEQ ID NO:2; Accession number gi:162330095); CNTN1 Isoform 2 of Contactin-1 (SEQ ID NO: 4; Accession Number gi:28373119); cDNA highly similar to SPARC-like protein 1 (unnamed protein product) (SEQ ID NO: 5; Accession Number: gi|194388050); NCAM2 Neural cell adhesion molecule 2, isoform CRA_a (SEQ ID NO: 8; Accession Number gi|119630409); SERPINA3 serpin peptidase inhibitor, clade A, member 3 precursor /Isoform 1 of Alpha-1-antichymotrypsin/growth-inhibiting protein 25 [Homo sapiens] or slightly longer fragment of alpha-1-antichymotrypsin precursor (SEQ ID NO: 9; Accession Number gi|46981961); AGT Angiotensinogen (SEQ ID NO: 10; Accession Number gi|553181); Angiotensinogen precursor (Serpina8) (SEQ ID NO: 11; Accession Number gi|4557287); unnamed protein product also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 12; Accession Number gi|187608363); SERPINF1 serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor, Pedf), member 1 isoform 4 factor (SEQ ID NO: 14; Accession Number gi|15988024); human protein similar to GC Vitamin D-binding protein PREDICTED: vitamin D-binding protein [Pan troglodytes] (SEQ ID NO: 15; Accession Number 181482); CD14 Human monocyte antigen CD14 (CD14) (SEQ ID NO: 16; Accession Number gi|117646212); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (SEQ ID NO: 17; Accession Number gi|90080503; SEQ ID NO: 18; gi|187608363 (human); Neural cell adhesion molecule variant (SEQ ID NO: 19; Accession Number gi:62088238); unnamed protein similar to CLU cDNA FLJ57622, highly similar to Clusterin (SEQ ID NO: 20; Accession number gi|189054091); protein highly similar to Clusterin (SEQ ID NO: 21; Accession number gi|193787502); LMAN2 Vesicular integral-membrane protein VIP36 (SEQ ID NO: 22; Accession number gi|157834800); clusterin isoform 1 [Homo sapiens] (SEQ ID NO: 23; Accession number NM_001831.2); superoxide dismutase 3, extracellular precursor (SEQ ID NO: 24; Accession number gi|118582275); fibrin alpha C term fragment (SEQ ID NO: 25; Accession number gi|223057); Chain A, Human Kallikrein 6 (Hk6) Active Form or KLK6 Isoform 1 of Kallikrein-6 (SEQ ID NO: 26; Accession number gi|21465970); APCS Serum amyloid P-component /Chain A or Pentameric Human Serum Amyloid P Component (SEQ ID NO: 27; Accession number gi|576259); FAM3C Protein

FAM3C / family with sequence similarity 3, member C precursor [Homo sapiens] note="predicted osteoblast protein; interleukin-like EMT inducer (SEQ ID NO: 28; Accession number gi|55629272); protein similar to unnamed protein product [Macaca fascicularis] also called immunoglobulin superfamily, member 4B; in humans, also called cell adhesion molecule 3 (SEQ ID NO: 29; Accession number gi|187608363); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched proteins.

- 5
10. The method of claim 8, further comprising contacting the sample with a binding partner specific for a second CSF-enriched protein, and
- 10 detecting the binding partner-second CSF-enriched protein complexes if present, wherein the presence of detectable complexes indicates the presence of said second CSF-enriched protein in the sample; wherein the second CSF-enriched protein is
- 15 CNTN2 Contactin-2 precursor (SEQ ID NO: 3; Accession Number gi|4827022); NRCAM protein (Neuronal cell adhesion molecule)[Homo sapiens] possibly slightly longer fragment (~96kDa) (SEQ ID NO: 6; Accession Number: gi|68534652 and SEQ ID NO: 7); CADM3 Homo sapiens cell adhesion molecule 3 (CADM3), transcript variant 1 (SEQ ID NO: 17); Neural cell adhesion molecule variant (SEQ ID NO: 19; Accession Number gi:62088238); or a CSF-enriched phosphorylated or dephosphorylated form of the foregoing CSF-enriched proteins.
- 20
11. The method of any one of claims 8-10, wherein the binding partner comprises a detectable label.
12. The method of any one of claims 8-11, further comprising quantitating the amount of binding partner-CSF-enriched protein complexes in the sample.
- 25
13. The method of any one of claims 8-10, wherein the binding partner is an antibody and detecting comprises (i) differential antibody binding or (ii) an *in situ* immunoassay.
14. The method of any one of claims 8-10, wherein the sample is tissue, blood, serum, plasma, urine, nasal and ear effluents, saliva, sweat, or tears, and/or wherein the sample is from an individual suspected of having a brain injury.
- 30
15. The method of any one of claims 8-10, wherein the binding partner is an antibody and (i) the method comprises contacting the sample with two to ten different antibodies that each specifically binds a different CSF-enriched protein, preferably wherein each antibody is employed at a subthreshold level, or (ii) the method comprises contacting the sample with four to ten different antibodies that each specifically binds a different CSF-enriched protein, and wherein a positive test does not require binding to all antibodies.
- 35
16. The method of any one of claims 8 to 15, wherein the binding partner is an antibody that binds a post-translational modification in the CSF-enriched protein.
- 40
17. The method of any one of claims 8 to 16, wherein the sample is suspected of containing CSF.

Patentansprüche

- 45
1. Verwendung einer Vorrichtung in einem *in vitro* Verfahren zum Nachweis der Anwesenheit oder Abwesenheit von Zerebrospinalflüssigkeit (CSF) in einer Probe, wobei das Verfahren umfasst
- Kontaktieren der Probe mit einem Bindungspartner, der für ein mit CSF angereichertes Protein spezifisch ist, und Nachweisen von mit Bindungspartner-CSF angereicherten Proteinkomplexen, sofern vorhanden, wobei die Anwesenheit von nachweisbaren Komplexen die Anwesenheit des mit CSF angereicherten Proteins in der Probe anzeigt;
- 50 wobei das mit CSF-angereicherte Protein cDNA FU59893, Dickkopf Homolog 3-Vorläufer (SEQ ID Nr.: 13; Hinterlegungsnummer gi|40548389); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form des vorstehend genannten mit CSF-angereicherten Proteins ist,
- wobei die Vorrichtung umfasst
- einen Probenapplizierbereich,
- 55 einen Probenetikettierbereich, umfassend einen ersten Antikörper gegen das mit CSF angereicherte Protein, wobei der erste Antikörper mit einem mobilen Partikel konjugiert ist;
- einen Probenachweisbereich, umfassend einen zweiten Antikörper gegen das mit CSF angereicherte Protein, wobei der zweite Antikörper an den Probenachweisbereich fixiert ist,

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wobei die Bildung eines nachweisbaren Bands im zweiten Bereich, im Nachgang zum Aufbringen der Probe auf den Probennachweisbereich, die Anwesenheit des mit CSF angereicherten Proteins in der Probe anzeigt.

2. Verwendung nach Anspruch 1, wobei die Vorrichtung zwei bis zehn verschiedene Antikörper umfasst, die jeweils spezifisch ein verschiedenes mit CSF angereichertes Protein binden.
3. Verwendung nach Anspruch 2, wobei die Vorrichtung ein einzelnes kombiniertes Ergebnis liefert, oder wobei die Vorrichtung ein individuelles Ergebnis für jeden Antikörper liefert, oder wobei jeder Antikörper auf einem Unterschwellenniveau eingesetzt wird.
4. Verwendung nach Anspruch 2, wobei die Vorrichtung vier bis zehn verschiedene Antikörper umfasst, die jeweils spezifisch ein verschiedenes mit CSF angereichertes Protein binden, und wobei ein positiver Test nicht die Bindung an alle Antikörper erfordert.
5. Verwendung nach einem der Ansprüche 1 bis 4, wobei der erste Antikörper gegen das mit CSF angereicherte Protein eine posttranslationale Modifikation in dem mit CSF angereicherten Protein bindet.
6. Verwendung nach einem der Ansprüche 2 bis 5, wobei die zwei bis zehn Antikörper ausgewählt sind aus
 - (i) einem Antikörper, der cDNA FU59893, Dickkopf Homolog 3-Vorläufer (SEQ ID Nr.: 13; Hinterlegungsnummer gi|40548389); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form desselben bindet; und
 - (ii) einem Antikörper, der einen CNTN2 Contactin-2-Vorläufer (SEQ ID Nr.: 3; Hinterlegungsnummer gi|4827022); NRCAM-Protein (neuronales Zelladhäsionsmolekül) [Homo sapiens], ein möglicherweise etwas längeres Fragment (~96 kDa) (SEQ ID Nr.: 6; Hinterlegungsnummer: gi|68534652 und SEQ ID Nr.: 7; Hinterlegungsnummer: gi|109731501); Isoform 1 eines neuralen Zelladhäsionsmolekül-ähnlichen Proteins (SEQ ID Nr.: 1; Hinterlegungsnummer gi:62088238); Kette A, humanes Mesotrypsin, komplexiert mit bovinem pankreatischem Trypsin-Inhibitor (Bpti) (SEQ ID Nr.: 2; Hinterlegungsnummer gi:162330095); CNTN1 Isoform 2 von Contactin-1 (SEQ ID Nr.: 4; Hinterlegungsnummer gi:28373119); cDNA, die sehr ähnlich ist wie SPARC-ähnliches Protein 1 (unbenanntes Proteinprodukt) (SEQ ID Nr.: 5; Hinterlegungsnummer: gi|194388050); NCAM2 neuronales Zelladhäsionsmolekül 2, Isoform CRA_a (SEQ ID Nr.: 8; Hinterlegungsnummer gi|119630409); SERPINA3 Serpin-Peptidase-Inhibitor, Klade A, Mitglied 3-Vorläufer /Isoform 1 von Alpha-1-Antichymotrypsin/wachstumshemmendes Protein 25 [Homo sapiens] oder ein etwas längeres Fragment von alpha-1-Antichymotrypsin-Vorläufer (SEQ ID Nr.: 9; Hinterlegungsnummer gi|46981961); AGT Angiotensinogen (SEQ ID Nr.: 10; Hinterlegungsnummer gi|553181); Angiotensinogen-Vorläufer (Serpin A8) (SEQ ID Nr.: 11; Hinterlegungsnummer gi 14557287); unbenanntes Proteinprodukt, auch Immunglobulin-Superfamilie genannt, Mitglied 4B; beim Menschen, auch Zelladhäsionsmolekül 3 genannt (SEQ ID Nr.: 12; Hinterlegungsnummer gi|187608363); ein SERPINF1 Serin (oder Cystein) Proteinaseinhibitor, Klade F (Alpha-2 Antiplasmin, Pigmentepithel-abgeleiteter Faktor, Pedf), Mitglied 1 Isoform 4 Faktor (SEQ ID Nr.: 14; Hinterlegungsnummer gi|15988024); humanes Protein ähnlich wie GC Vitamin D-Bindungsprotein PROGNOSTIZIERT: Vitamin D-Bindungsprotein [Pan troglodytes] (SEQ ID Nr.: 15; Hinterlegungsnummer 181482); CD14 humanes Monozytenantigen CD14 (CD 14) (SEQ ID Nr.: 16; Hinterlegungsnummer gi|117646212); CADM3 Homo sapiens Zelladhäsionsmolekül 3 (CADM3), Transkriptvariante 1 (SEQ ID Nr.: 17; Hinterlegungsnummer gi|90080503; SEQ ID Nr.: 18; gi|187608363 (human); neurale Zelladhäsionsmolekülvariante (SEQ ID Nr.: 19; Hinterlegungsnummer gi:62088238); unbenanntes Protein, ähnlich wie CLU cDNA FU57622, sehr ähnlich wie Clusterin (SEQ ID Nr.: 20; Hinterlegungsnummer gi|189054091); Protein, sehr ähnlich wie Clusterin (SEQ ID Nr.: 21; Hinterlegungsnummer gi|193787502); LMAN2 vesikuläres Integralmembranprotein VIP36 (SEQ ID Nr.: 22; Hinterlegungsnummer gi|157834800); Clusterin Isoform 1 [Homo sapiens] (SEQ ID Nr.: 23; Hinterlegungsnummer NM_001831.2); Superoxiddismutase 3, extrazellulärer Vorläufer (SEQ ID Nr.: 24; Hinterlegungsnummer gi|118582275); Fibrin-alpha-C-Stadiumfragment (SEQ ID Nr.: 25; Hinterlegungsnummer gi|223057); Kette A, humanes Kallikrein 6 (Hk6) aktive Form oder KLK6 Isoform 1 von Kallikrein-6 (SEQ ID Nr.: 26; Hinterlegungsnummer gi|21465970); APCS Serum Amyloid P-Komponente /Kette A oder pentamerische humane Serum Amyloid P-Komponente (SEQ ID Nr.: 27; Hinterlegungsnummer gi|576259); FAM3C Protein FAM3C / Familie mit Sequenzähnlichkeit 3, Mitglied C-Vorläufer [Homo sapiens] Hinweis="prognostizierter Osteoblastprotein; Interleukin-ähnlicher EMT Induktor (SEQ ID Nr.: 28; Hinterlegungsnummer gi|55629272); Protein ähnlich wie unbenanntes Proteinprodukt [Macaca fascicularis], auch Immunglobulin-Superfamilie genannt, Mitglied 4B; beim Menschen, auch Zelladhäsionsmolekül 3 genannt (SEQ ID Nr.: 29; Hinterlegungsnummer gi |187608363); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form der vorstehend genannten mit

CSF angereicherten Proteine bindet.

7. Verwendung nach einem der Ansprüche 2 bis 5, wobei die zwei bis zehn verschiedenen Antikörper ausgewählt sind aus

(i) einem Antikörper, der cDNA FU59893, Dickkopf Homolog 3-Vorläufer (SEQ ID Nr.: 13; Hinterlegungsnummer gj|40548389); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form desselben bindet; und

(ii) einem Antikörper, der CNTN2 Contactin-2-Vorläufer (SEQ ID Nr.: 3; Hinterlegungsnummer gj|4827022); NRCAM-Protein (Neuronales Zelladhäsionsmolekül) [Homo sapiens], ein möglicherweise etwas längeres Fragment (~96 kDa) (SEQ ID Nr.: 6; Hinterlegungsnummer: gj|68534652 und SEQ ID Nr.: 7); CADM3 Homo sapiens Zelladhäsionsmolekül 3 (CADM3), Transkriptvariante 1 (SEQ ID Nr.: 17); neurale Zelladhäsionsmolekülvariante (SEQ ID Nr.: 19; Hinterlegungsnummer gi:62088238); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form der vorstehend genannten mit CSF angereicherten Proteine bindet.

8. *In vitro* Verfahren zum Nachweis der Anwesenheit oder Abwesenheit von CSF in einer Probe, wobei das Verfahren umfasst

Kontaktieren der Probe mit einem Bindungspartner, der für ein mit CSF angereichertes Protein spezifisch ist, und Nachweisen von mit Bindungspartner-CSF angereicherten Proteinkomplexen, sofern vorhanden, wobei die Anwesenheit von nachweisbaren Komplexen die Anwesenheit des mit CSF angereicherten Proteins in der Probe anzeigt; wobei das mit CSF angereicherte Protein cDNA FU59893, Dickkopf Homolog 3-Vorläufer (SEQ ID Nr.: 13; Hinterlegungsnummer gj|40548389); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form des vorstehend genannten mit CSF angereicherten Proteins ist.

9. Verfahren nach Anspruch 8, weiter umfassend

Kontaktieren der Probe mit einem Bindungspartner, der für ein zweites mit CSF angereichertes Protein spezifisch ist, und

Nachweisen der mit Bindungspartner-zweiten-CSF angereicherten Proteinkomplexe, sofern vorhanden, wobei die Anwesenheit von nachweisbaren Komplexen die Anwesenheit des mit zweiten CSF angereicherten Proteins in der Probe anzeigt;

wobei das mit zweitem CSF angereicherte Protein

CNTN2 Contactin-2-Vorläufer (SEQ ID Nr.: 3; Hinterlegungsnummer gj|4827022); NRCAM-Protein (neuronalen Zelladhäsionsmolekül) [Homo sapiens], ein möglicherweise etwas längeres Fragment (~96 kDa) (SEQ ID Nr.: 6; Hinterlegungsnummer: gj|68534652 und SEQ ID Nr.: 7; Hinterlegungsnummer: gj|109731501); Isoform 1 des neuronalen Zelladhäsionsmolekül-ähnlichen Proteins (SEQ ID Nr.: 1; Hinterlegungsnummer gi:62088238); Kette A, humanes Mesotrypsin, komplexiert mit bovinem pankreatischem Trypsin-Inhibitor (Bpti) (SEQ ID Nr.: 2; Hinterlegungsnummer gi:162330095); CNTN1 Isoform 2 von Contactin-1 (SEQ ID Nr.: 4; Hinterlegungsnummer gi:28373119); cDNA, die sehr ähnlich ist wie SPARC-ähnliches Protein 1 (unbenanntes Proteinprodukt) (SEQ ID Nr.: 5; Hinterlegungsnummer: gj|194388050); NCAM2 neuronales Zelladhäsionsmolekül 2, Isoform CRA_a (SEQ ID Nr.: 8; Hinterlegungsnummer gj|119630409); SERPINA3 Serpin-Peptidaseinhibitor, Klade A, Mitglied 3-Vorläufer /Isoform 1 von Alpha-1-Antichymotrypsin/wachstumshemmendes Protein 25 [Homo sapiens] oder ein etwas längeres Fragment des Alpha-1-Antichymotrypsin-Vorläufers (SEQ ID Nr.: 9; Hinterlegungsnummer gj|46981961); AGT Angiotensinogen (SEQ ID Nr.: 10; Hinterlegungsnummer gj|553181); Angiotensinogen-Vorläufer (Serpin A8) (SEQ ID Nr.: 11; Hinterlegungsnummer gj|4557287); ungenanntes Proteinprodukt, auch Immunoglobulin-Superfamilie genannt, Mitglied 4B; beim Menschen, auch Zelladhäsionsmolekül 3 genannt (SEQ ID Nr.: 12; Hinterlegungsnummer gj|187608363); SERPINF1 Serin (oder Cystein) Proteinase-Inhibitor, Klade F (Alpha-2 Antiplasmin, Pigmentepithel-abgeleiteter Faktor, Pedf), Mitglied 1 Isoform 4 Faktor (SEQ ID Nr.: 14; Hinterlegungsnummer gj|15988024); humanes Protein ähnlich wie GC Vitamin D-Bindungsprotein PROGNOSTIZIERT: Vitamin D-Bindungsprotein [Pan troglodytes] (SEQ ID Nr.: 15; Hinterlegungsnummer 181482); CD14 humanes Monozyten-Antigen CD14 (CD14) (SEQ ID Nr.: 16; Hinterlegungsnummer gj|117646212); CADM3 Homo sapiens Zelladhäsionsmolekül 3 (CADM3), Transkriptvariante 1 (SEQ ID Nr.: 17; Hinterlegungsnummer gj|90080503; SEQ ID Nr.: 18; gj|187608363 (human); neurale Zelladhäsionsmolekülvariante (SEQ ID Nr.: 19; Hinterlegungsnummer gj|62088238); unbenanntes Protein ähnlich wie CLU cDNA FU57622, sehr ähnlich wie Clusterin (SEQ ID Nr.: 20; Hinterlegungsnummer gj|189054091); Protein sehr ähnlich wie Clusterin (SEQ ID Nr.: 21; Hinterlegungsnummer gj|193787502); LMAN2 vesikuläres Integralmembranprotein VIP36 (SEQ ID Nr.: 22; Hinterlegungsnummer gj|157834800); Clusterin Isoform 1 [Homo sapiens] (SEQ ID Nr.: 23; Hinterlegungsnummer NM_001831.2); Superoxiddismutase 3, extrazellulärer-Vorläufer (SEQ ID Nr.: 24; Hinterlegungsnummer gj|118582275); Fibrin alpha C-Stadiumfragment (SEQ ID Nr.: 25; Hinterlegungsnummer gj|223057); Kette A, humanes Kallikrein 6 (Hk6) aktive Form oder KLK6 Isoform 1 von Kallikrein-6 (SEQ ID Nr.: 26;

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Hinterlegungsnummer gi|21465970); APCS Serum Amyloid P-Komponente /Kette A oder pentamerisches humanes Serum Amyloid P-Komponente (SEQ ID Nr.: 27; Hinterlegungsnummer gi|576259); FAM3C Protein FAM3C/ Familie mit Sequenzähnlichkeit 3, Mitglied C-Vorläufer [Homo sapiens] Hinweis="prognostizierter Osteoblastprotein; Interleukin-ähnlicher EMT Induktor (SEQ ID Nr.: 28; Hinterlegungsnummer gi|55629272); Protein, ähnlich wie unbenanntes Proteinprodukt [Macaca fascicularis], auch Immunglobulin-Superfamilie genannt, Mitglied 4B; beim Menschen, auch Zelladhäsionsmolekül 3 genannt (SEQ ID Nr.: 29; Hinterlegungsnummer gi|187608363); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form der vorstehend genannten mit CSF angereicherten Proteine ist.

10. Verfahren nach Anspruch 8, weiter umfassend

Kontaktieren der Probe mit einem Bindungspartner, der für ein zweites mit CSF angereichertes Protein spezifisch ist, und

Nachweisen der mit Bindungspartner-zweiten-CSF angereicherten Proteinkomplexe, sofern vorhanden, wobei die Anwesenheit von nachweisbaren Komplexen die Anwesenheit des mit zweiten CSF angereicherten Proteins in der Probe anzeigt;

wobei das mit zweiten CSF angereicherte Protein

CNTN2 Contactin-2-Vorläufer (SEQ ID Nr.: 3; Hinterlegungsnummer gi|14827022); NRCAM-Protein (neurales Zelladhäsionsmolekül) [Homo sapiens], ein möglicherweise etwas längeres Fragment (~96 kDa) (SEQ ID Nr.: 6; Hinterlegungsnummer: gi|68534652 und SEQ ID Nr.: 7); CADM3 Homo sapiens Zelladhäsionsmolekül 3 (CADM3), Transkriptvariante 1 (SEQ ID Nr.: 17); neurale Zelladhäsionsmolekülvariante (SEQ ID Nr.: 19; Hinterlegungsnummer gi:62088238); oder eine mit CSF angereicherte phosphorylierte oder dephosphorylierte Form der vorstehend genannten mit CSF angereicherten Proteine ist.

11. Verfahren nach einem der Ansprüche 8 bis 10, wobei der Bindungspartner ein nachweisbares Etikett umfasst.

12. Verfahren nach einem der Ansprüche 8 bis 11, weiter umfassend Quantifizieren der Menge von mit Bindungspartner-CSF angereicherter Proteinkomplexe in der Probe.

13. Verfahren nach einem der Ansprüche 8 bis 10, wobei der Bindungspartner ein Antikörper ist und das Nachweisen (i) eine differentielle Antikörperbindung oder (ii) einen in situ Immunoassay umfasst.

14. Verfahren nach einem der Ansprüche 8 bis 10, wobei die Probe Gewebe, Blut, Serum, Plasma, Urin, Nasen- und Ohrenausfluss, Speichel, Schweiß oder Tränen ist, und/oder wobei die Probe von einem Individuum stammt, das im Verdacht steht, eine Hirnverletzung aufzuweisen.

15. Verfahren nach einem der Ansprüche 8 bis 10, wobei der Bindungspartner ein Antikörper ist und (i) das Verfahren das Kontaktieren der Probe mit zwei bis zehn verschiedenen Antikörpern umfasst, die jeweils spezifisch an ein verschiedenes mit CSF angereichertes Protein binden, vorzugsweise wobei jeder Antikörper auf einem Unterschwellenniveau eingesetzt wird, oder (ii) wobei das Verfahren das Kontaktieren der Probe mit vier bis zehn verschiedenen Antikörpern umfasst, die jeweils spezifisch an ein anderes mit CSF angereichertes Protein binden, und wobei ein positiver Test keine Bindung an alle Antikörper erfordert.

16. Verfahren nach einem der Ansprüche 8 bis 15, wobei der Bindungspartner ein Antikörper ist, der eine posttranslationale Modifikation in dem mit CSF angereicherten Protein bindet.

17. Verfahren nach einem der Ansprüche 8 bis 16, wobei die Probe im Verdacht steht, CSF zu enthalten.

Revendications

1. Utilisation d'un dispositif dans un procédé in vitro pour détecter la présence ou l'absence de liquide céphalorachidien (LCR) dans un échantillon, ledit procédé comprenant la mise en contact de l'échantillon avec un partenaire de liaison spécifique pour une protéine enrichie en LCR, et la détection de complexes protéine enrichie en LCR-partenaire de liaison, si présents, dans laquelle la présence de complexes détectables indique la présence de ladite protéine enrichie en LCR dans l'échantillon ; dans laquelle la protéine enrichie en LCR est le précurseur d'homologue 3 de Dickkopf, ADNc FLJ59893, (SEQ ID n° 13 ; numéro d'accès gi|40548389) ; ou une forme phosphorylée ou déphosphorylée enrichie en LCR de la protéine enrichie en LCR précédente,

ledit dispositif comprenant

une région d'application d'échantillon,

une région d'étiquetage d'échantillon comprenant un premier anticorps à ladite protéine enrichie en LCR, dans laquelle le premier anticorps est conjugué à une particule mobile ;

une région de détection d'échantillon comprenant un second anticorps à ladite protéine enrichie en LCR, dans laquelle le second anticorps est fixé à la région de détection d'échantillon,

dans laquelle la formation d'une bande détectable dans la seconde région après l'application de l'échantillon à la région d'application d'échantillon indique la présence de ladite protéine enrichie en LCR dans l'échantillon.

2. Utilisation selon la revendication 1, dans laquelle le dispositif comprend deux à dix anticorps différents qui se lient chacun de manière spécifique à une protéine différente enrichie en LCR.

3. Utilisation selon la revendication 2, dans laquelle le dispositif fournit un résultat combiné unique, ou le dispositif fournit un résultat individuel pour chaque anticorps, ou dans lequel chaque anticorps est utilisé à un niveau de sous-seuil.

4. Utilisation selon la revendication 2, dans laquelle le dispositif comprend quatre à dix anticorps différents qui se lient chacun de manière spécifique à une protéine différente enrichie en LCR, et dans laquelle un test positif n'exige pas de liaison à tous les anticorps.

5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le premier anticorps à ladite protéine enrichie en LCR se lie à une modification post-translationnelle dans la protéine enrichie en LCR.

6. Utilisation selon l'une quelconque des revendications 2 à 5, dans laquelle les deux à dix anticorps sont sélectionnés parmi

(i) un anticorps qui se lie au précurseur d'homologue 3 de Dickkopf, ADNc FLJ59893, (SEQ ID n° 13 ; numéro d'accès gi |40548389) ; ou une forme phosphorylée ou déphosphorylée enrichie en LCR de celui-ci ; et

(ii) un anticorps qui se lie au précurseur de Contactine 2 CNTN2 (SEQ ID n° 3 ; numéro d'accès gi|4827022) ;

à un fragment potentiellement légèrement plus long (~96 kDa) de la protéine NRCAM (molécule d'adhésion de cellules neuronales) [Homo sapiens] (SEQ ID n° 6 ; numéro d'accès: gi|68534652 et SEQ ID n°7 ; numéro d'accès: gi|109731501) ; à l'Isoforme 1 d'une protéine semblable à une molécule d'adhésion de cellules neuro-

nales (SEQ ID n° 1 ; numéro d'accès gi:62088238) ; à la Chaîne A, Mésotrypsine Humaine Complexée Avec l'Inhibiteur de Trypsine Pancréatique Bovine (Bpti) (SEQ ID n° 2 ; numéro d'accès gi:162330095) ; à l'Isoforme 2 de Contactine 1 CNTN1 (SEQ ID n° 4 ; numéro d'accès gi:28373119) ; à l'ADNc hautement similaire à la

protéine 1 semblable à SPARC (produit protéinique anonyme) (SEQ ID n° 5 ; numéro d'accès : gi:194388050) ; à l'isoforme CRA_a, molécule d'adhésion de cellules Neuronales 2 NCAM2, (SEQ ID n° 8 ; numéro d'accès gi

|119630409) ; à l'inhibiteur de serpine peptidase SERPINA3, Clade A, précurseur d'élément 3 / Isoforme 1 d'Alpha-1-antichymotrypsine / protéine d'inhibition de croissance 25 [Homo sapiens] ou un fragment légèrement

plus long de précurseur d'alpha-1-antichymotrypsine (SEQ ID n° 9 ; numéro d'accès gi|46981961) ; à l'Angio-

tensinogène AGT (SEQ ID n° 10 ; numéro d'accès gi|553181) ; au précurseur d'Angiotensinogène (Serpine A8) (SEQ ID n° 11 ; numéro d'accès gi|4557287) ; à un produit protéinique anonyme également appelé super-

famille d'immunoglobuline, élément 4B ; chez les humains, également appelé molécule d'adhésion cellulaire 3 (SEQ ID n° 12 ; numéro d'accès gi|187608363) ; à l'inhibiteur de sérine (ou cystéine) protéinase SERPINF1,

Clade F (alpha 2 antiplasmine, facteur dérivé d'épithélium pigmentaire, Pedf), facteur d'élément 1 isoforme 4 (SEQ ID n° 14 ; numéro d'accès gi |15988024) ; à la protéine humaine similaire à la protéine de liaison à la

Vitamine D GC PRÉVUE : protéine de liaison à la vitamine D [Pan troglodytes] (SEQ ID n° 15 ; numéro d'accès 181482) ; à un antigène CD14 de monocytes Humains CD14 (CD14) (SEQ ID n° 16 ; numéro d'accès

gi|117646212) ; à un variant de transcription 1 d'une molécule d'adhésion cellulaire d'Homo sapiens 3 CADM3 (CADM3), (SEQ ID n° 17 ; numéro d'accès gi|90080503 ; SEQ ID n° 18 ; gi|187608363 (humain) ; à un variant

de molécule d'adhésion de cellules neuronales (SEQ ID n° 19 ; numéro d'accès gi:62088238) ; à une protéine anonyme similaire à l'ADNc CLU FU57622, hautement similaire à la Clusterine (SEQ ID n° 20 ; numéro d'accès

gi|189054091) ; à une protéine hautement similaire à la Clusterine (SEQ ID n° 21 ; numéro d'accès gi|193787502) ; à la protéine de membrane Vésiculaire entière VIP36 LMAN2 (SEQ ID n° 22 ; numéro d'accès

gi|157834800) ; à l'isoforme 1 de la clusterine [Homo sapiens] (SEQ ID n° 23 ; numéro d'accès NM_001831.2) ; au précurseur extracellulaire de superoxyde dismutase 3, (SEQ ID n° 24 ; numéro d'accès gi|118582275) ; au

fragment de terminaison C de fibrine alpha (SEQ ID n° 25 ; numéro d'accès gi|223057) ; à la Forme Active de Kallibréine Humaine 6 (Hk6), Chaîne A, ou à l'isoforme 1 de la Kallibréine 6 KLK6 (SEQ ID n° 26 ; numéro

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d'accès gi|21465970) ; au composant P d'amyloïde Sérique APCS / Composant P d'Amyloïde Sérique Humain Pentamérique ou de Chaîne A (SEQ ID n° 27; numéro d'accès gi|576259) ; à la FAM3C Protéine FAM3C / la famille avec une similitude de séquence 3, précurseur d'élément C [Homo sapiens] note = « protéine d'ostéoblaste prévue; à l'inducteur EMT semblable à l'interleukine (SEQ ID n° 28 ; numéro d'accès gi|55629272) ; à une protéine similaire à un produit protéinique anonyme [Macaca fascicularis] également appelé superfamille d'immunoglobuline, l'élément 4B ; chez les humains, également appelée molécule d'adhésion cellulaire 3 (SEQ ID n° 29; numéro d'accès gi |187608363) ; ou à une forme phosphorylée ou déphosphorylée enrichie en LCR des protéines précédentes enrichies en LCR.

7. Utilisation selon l'une quelconque des revendications 2 à 5, dans laquelle les deux à dix anticorps différents sont sélectionnés parmi

(i) un anticorps qui se lie au précurseur d'homologue 3 de Dickkopf, ADNc FLJ59893, (SEQ ID n° 13 ; numéro d'accès gi |40548389) ; ou à une forme phosphorylée ou déphosphorylée enrichie en LCR de celui-ci ; et
(ii) un anticorps qui se lie au précurseur de Contactine 2 CNTN2 (SEQ ID n° 3 ; numéro d'accès gi|4827022); à un fragment potentiellement légèrement plus long (~96 kDa) de la protéine NRCAM (molécule d'adhésion de cellules neuronales) [Homo sapiens] (SEQ ID n° 6 ; numéro d'accès : gi|68534652 et SEQ ID n° 7) ; à un variant de transcription 1 de la molécule 3 d'adhésion cellulaire d'Homo sapiens CADM3 (CADM3), (SEQ ID n° 17) ; à un variant de molécule d'adhésion de cellules neuronales (SEQ ID n° 19 ; numéro d'accès gi:62088238) ; ou à une forme phosphorylée ou déphosphorylée enrichie en LCR des protéines précédentes enrichies en LCR.

8. Procédé *in vitro* pour détecter la présence ou l'absence de LCR dans un échantillon, ledit procédé comprenant la mise en contact de l'échantillon avec un partenaire de liaison spécifique pour une protéine enrichie en LCR, et la détection de complexes protéine enrichie en LCR-partenaire de liaison, si présents, dans lequel la présence de complexes détectables indique la présence de ladite protéine enrichie en LCR dans l'échantillon ; dans lequel la protéine enrichie en LCR est le précurseur d'homologue 3 de Dickkopf, ADNc FLJ59893, (SEQ ID n° 13 ; numéro d'accès gi |40548389) ; ou une forme phosphorylée ou déphosphorylée enrichie en LCR de la protéine enrichie en LCR précédente.

9. Procédé selon la revendication 8, comprenant en outre la mise en contact de l'échantillon avec un partenaire de liaison spécifique pour une seconde protéine enrichie en LCR, et

la détection des complexes seconde protéine enrichie en LCR-partenaire de liaison, si présents, dans lequel la présence de complexes détectables indique la présence de ladite seconde protéine enrichie en LCR dans l'échantillon ;

dans lequel la seconde protéine enrichie en LCR est

le précurseur de Contactine 2 CNTN2 (SEQ ID n° 3 ; numéro d'accès gi|4827022) ; un fragment potentiellement légèrement plus long (~96 kDa) de la protéine NRCAM (molécule d'adhésion de cellules neuronales) [Homo sapiens] (SEQ ID n° 6 ; numéro d'accès: gi|68534652 et SEQ ID n° 7; numéro d'accès: gi|109731501) ; l'Isoforme 1 d'une protéine semblable à une molécule d'adhésion de cellules neuronales (SEQ ID n° 1 ; numéro d'accès gi:62088238) ; la Chaîne A, Mésotrypsine Humaine Complexée Avec l'Inhibiteur de Trypsine Pancréatique Bovine (Bpti) (SEQ ID n° 2 ; numéro d'accès gi:162330095) ; l'Isoforme 2 de Contactine 1 CNTN1 (SEQ ID n° 4 ; numéro d'accès gi:28373119) ; l'ADNc hautement similaire à la protéine 1 semblable à SPARC (produit protéinique anonyme) (SEQ ID n° 5 ; numéro d'accès : gi:194388050) ; l'isoforme CRA_a, molécule d'adhésion de cellules Neuronales 2 NCAM2, (SEQ ID n° 8 ; numéro d'accès gi|119630409) ; l'inhibiteur de serpine peptidase SERPINA3, Clade A, précurseur d'élément 3 / Isoforme 1 d'Alpha-1-antichymotrypsine / protéine d'inhibition de croissance 25 [Homo sapiens] ou un fragment légèrement plus long de précurseur d'alpha-1-antichymotrypsine (SEQ ID n° 9 ; numéro d'accès gi|46981961) ; l'Angiotensinogène AGT(SEQ ID n° 10; numéro d'accès gi|553181) ; le précurseur d'Angiotensinogène (Serpine A8) (SEQ ID n° 11 ; numéro d'accès gi|4557287) ; un produit protéinique anonyme également appelé superfamille d'immunoglobuline, élément 4B ; chez les humains, également appelé molécule d'adhésion cellulaire 3(SEQ ID n° 12; numéro d'accès gi|187608363) ; l'inhibiteur de sérine (ou cystéine) protéinase SERPINF1, Clade F (alpha 2 antiplasme, facteur dérivé d'épithélium pigmentaire, Pedf), facteur d'élément 1 isoforme 4 (SEQ ID n° 14; numéro d'accès gi|15988024) ; la protéine humaine similaire à la protéine de liaison à la Vitamine D GC PRÉVUE: protéine de liaison à la vitamine D [Pan troglodytes] (SEQ ID n° 15 ; numéro d'accès 181482) ; un antigène CD14 de monocytes Humains CD14 (CD14) (SEQ ID n° 16 ; numéro d'accès gi|117646212) ; un variant de transcription 1 d'une molécule d'adhésion cellulaire d'Homo sapiens 3 CADM3 (CADM3), (SEQ ID n° 17 ; numéro d'accès gi|90080503; SEQ ID n° 18 ; gi|187608363 (humain) ; un variant de molécule d'adhésion de cellules neuronales (SEQ ID n° 19 ; numéro d'accès gi:62088238) ; une protéine anonyme similaire à l'ADNc CLU FU57622, hautement

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similaire à la Clusterine (SEQ ID n° 20 ; numéro d'accès gi|189054091) ; une protéine hautement similaire à la Clusterine (SEQ ID n° 21 ; numéro d'accès gi|193787502) ; la protéine de membrane Vésiculaire entière VIP36 LMAN2 (SEQ ID n° 22 ; numéro d'accès gi|157834800) ; l'isoforme 1 de la clusterine [Homo sapiens] (SEQ ID n° 23 ; numéro d'accès NM_001831.2) ; le précurseur extracellulaire de superoxyde dismutase 3, (SEQ ID n° 24 ;
5 numéro d'accès gi|118582275) ; un fragment de terminaison C de fibrine alpha (SEQ ID n° 25 ; numéro d'accès gi|223057) ; la Forme Active de Kallicréine Humaine 6 (Hk6), Chaîne A, ou l'Isoforme 1 de la Kallicréine 6 KLK6 (SEQ ID n° 26 ; numéro d'accès gi|21465970) ; le composant P d'amyloïde Sérique APCS / Composant P d'Amyloïde Sérique Humain Pentamérique ou de Chaîne A (SEQ ID n° 27 ; numéro d'accès gi|576259) ; la FAM3C Protéine FAM3C / la famille avec une similitude de séquence 3, précurseur d'élément C [Homo sapiens] note = « protéine
10 d'ostéoblaste prévue ; l'inducteur EMT semblable à l'interleukine (SEQ ID n° 28 ; numéro d'accès gi|55629272) ; une protéine similaire à un produit protéinique anonyme [Macaca fascicularis] également appelé superfamille d'immunoglobuline, l'élément 4B ; chez les humains, également appelée molécule d'adhésion cellulaire 3 (SEQ ID n° 29 ; numéro d'accès gi|187608363) ; ou à une forme phosphorylée ou déphosphorylée enrichie en LCR des protéines
15 précédentes enrichies en LCR.

10. Procédé selon la revendication 8, comprenant en outre
la mise en contact de l'échantillon avec un partenaire de liaison spécifique pour une seconde protéine enrichie en LCR,
et
20 la détection des complexes seconde protéine enrichie en LCR-partenaire de liaison, si présents, dans lequel la présence de complexes détectables indique la présence de ladite seconde protéine enrichie en LCR dans l'échantillon ;
dans lequel la seconde protéine enrichie en LCR est
le précurseur de Contactine 2 CNTN2 (SEQ ID n° 3 ; numéro d'accès gi|4827022) ; un fragment potentiellement
25 légèrement plus long (~96 kDa) de la protéine NRCAM (molécule d'adhésion de cellules neuronales) [Homo sapiens] (SEQ ID n° 6 ; numéro d'accès: gi|68534652 et SEQ ID n°7) ; un variant de transcription 1 de la molécule 3 d'adhésion cellulaire d'Homo sapiens CADM3 (CADM3), (SEQ ID n° 17) ; un variant de molécule d'adhésion de cellules neuronales (SEQ ID n° 19 ; numéro d'accès gi:62088238) ; ou à une forme phosphorylée ou déphosphorylée enrichie en LCR des protéines précédentes enrichies en LCR.
30
11. Procédé selon l'une quelconque des revendications 8 à 10, dans lequel le partenaire de liaison comprend une étiquette détectable.
12. Procédé selon l'une quelconque des revendications 8 à 11, comprenant en outre la quantification de la quantité de
35 complexes protéine enrichie en LCR-partenaire de liaison dans l'échantillon.
13. Procédé selon l'une quelconque des revendications 8 à 10, dans lequel le partenaire de liaison est un anticorps et la détection comprend (i) la liaison différentielle d'un anticorps ou (ii) un immuno-essai *in situ*.
- 40 14. Procédé selon l'une quelconque des revendications 8 à 10, dans lequel l'échantillon est du tissu, du sang, du sérum, du plasma, de l'urine, des effluents nasaux et auriculaires, de la salive, de la sueur, ou des larmes, et/ou dans lequel l'échantillon provient d'un individu soupçonné d'avoir une lésion cérébrale.
- 45 15. Procédé selon l'une quelconque des revendications 8 à 10, dans lequel le partenaire de liaison est un anticorps et (i) le procédé comprend la mise en contact de l'échantillon avec deux à dix anticorps différents qui se lient chacun de manière spécifique à une protéine enrichie en LCR différente, de préférence dans lequel chaque anticorps est utilisé à un niveau de sous-seuil, ou (ii) le procédé comprend la mise en contact de l'échantillon avec quatre à dix anticorps différents qui se lient chacun de manière spécifique à une protéine enrichie en LCR différente, et dans lequel un test positif n'exige pas de liaison à tous les anticorps.
50
16. Procédé selon l'une quelconque des revendications 8 à 15, dans lequel le partenaire de liaison est un anticorps qui se lie à une modification post-translacionnelle dans la protéine enrichie en LCR.
- 55 17. Procédé selon l'une quelconque des revendications 8 à 16, dans lequel l'échantillon est soupçonné de contenir du LCR.

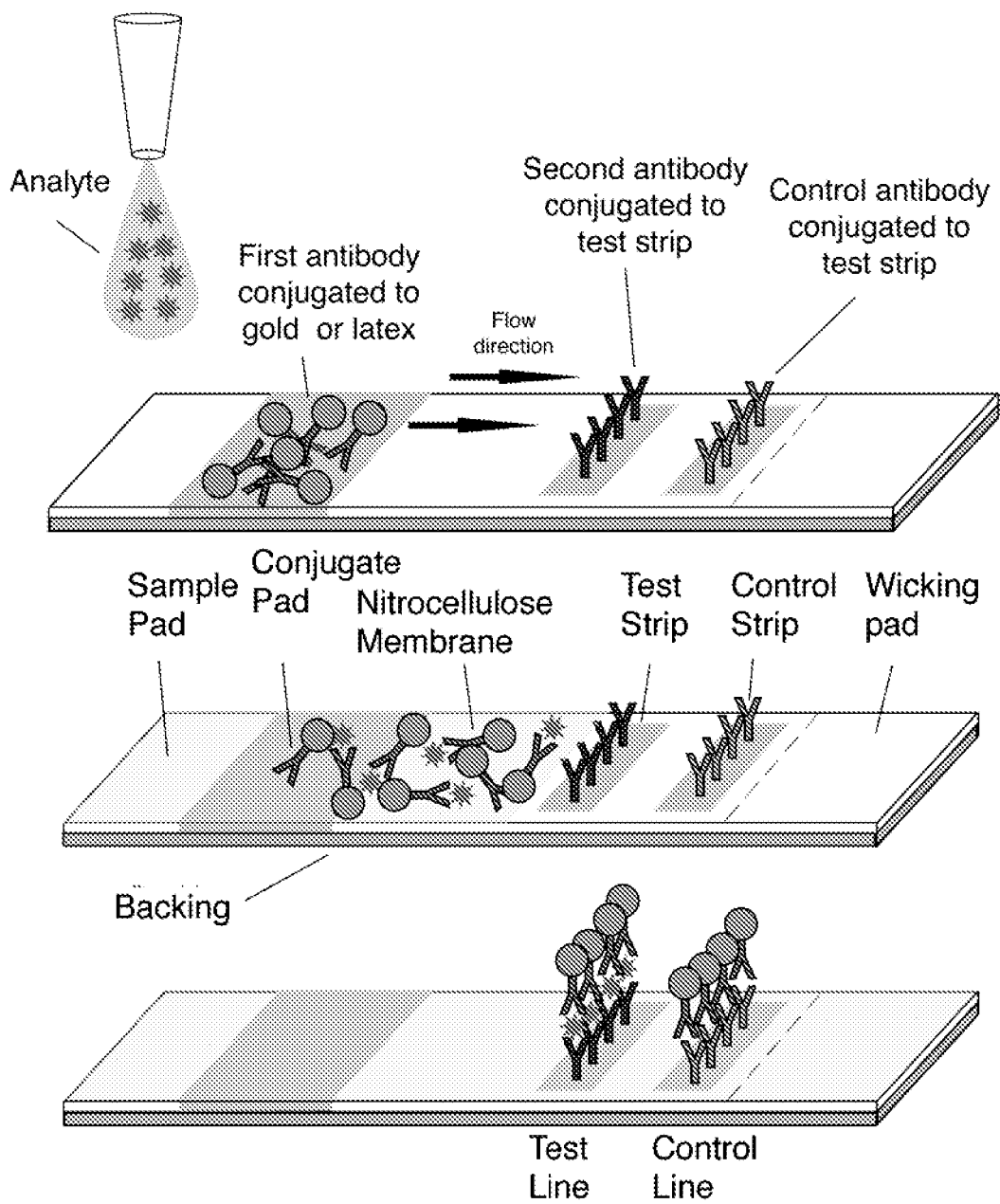


Figure 1

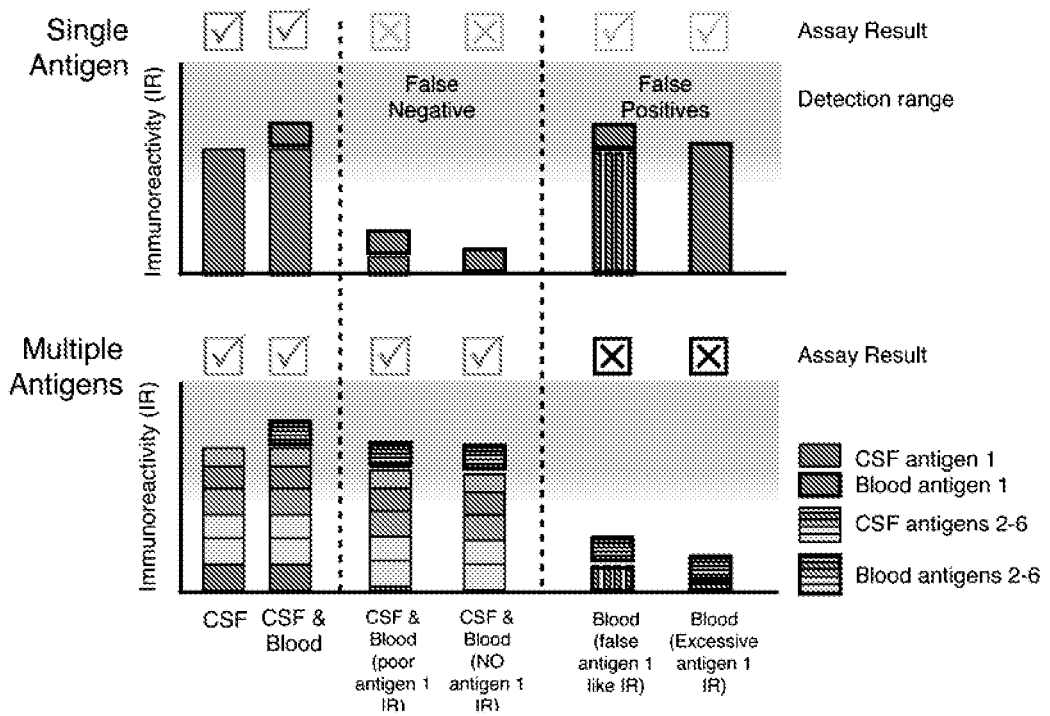


Figure 2

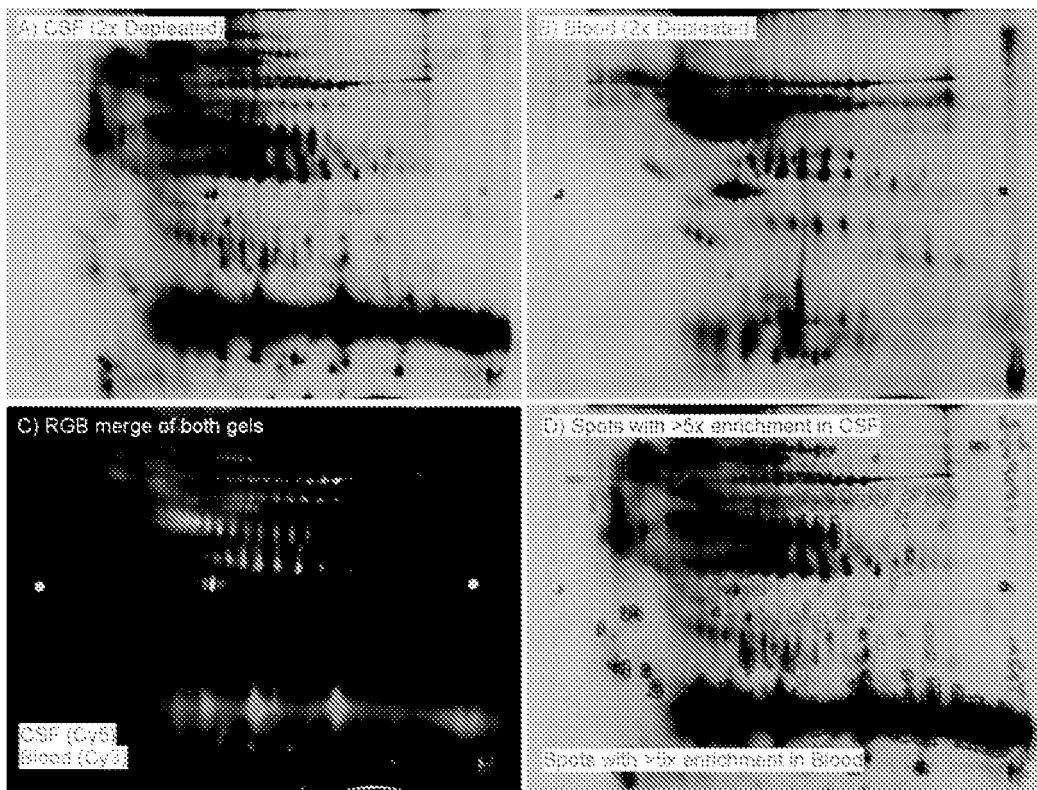


Figure 3

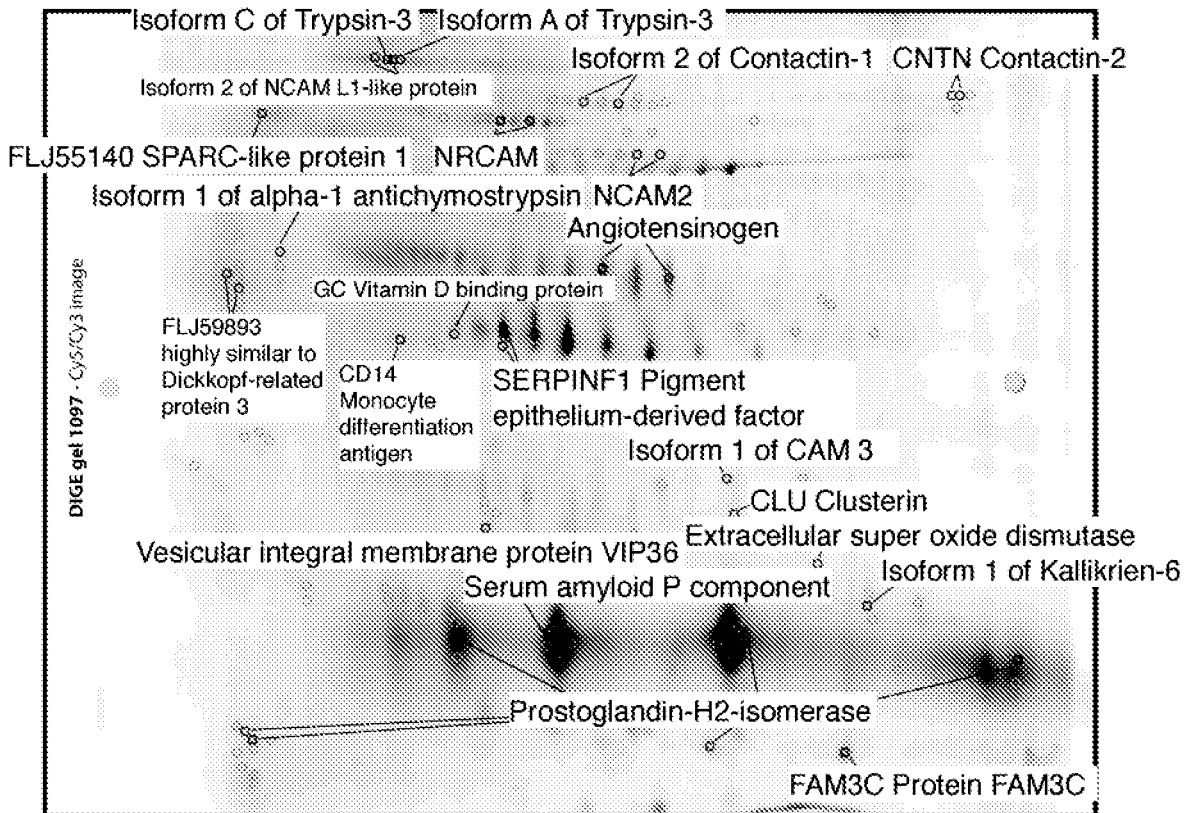


Figure 4

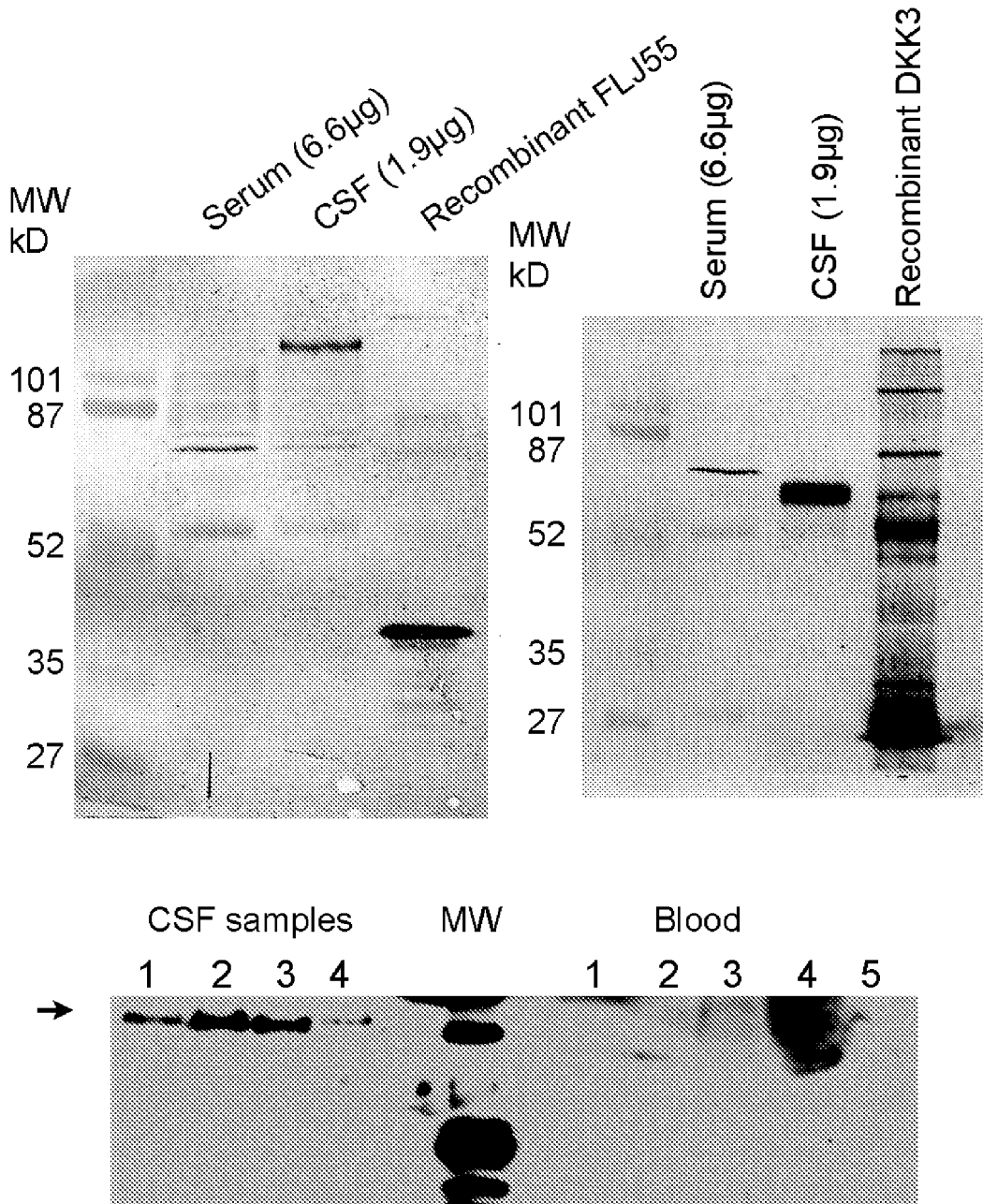


Figure 5

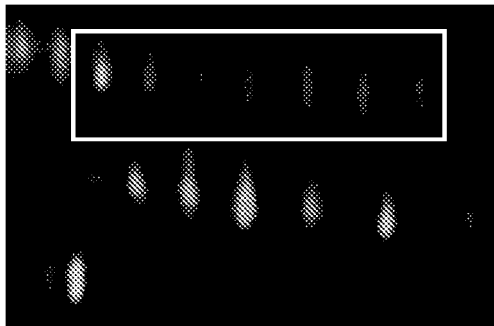


Figure 6

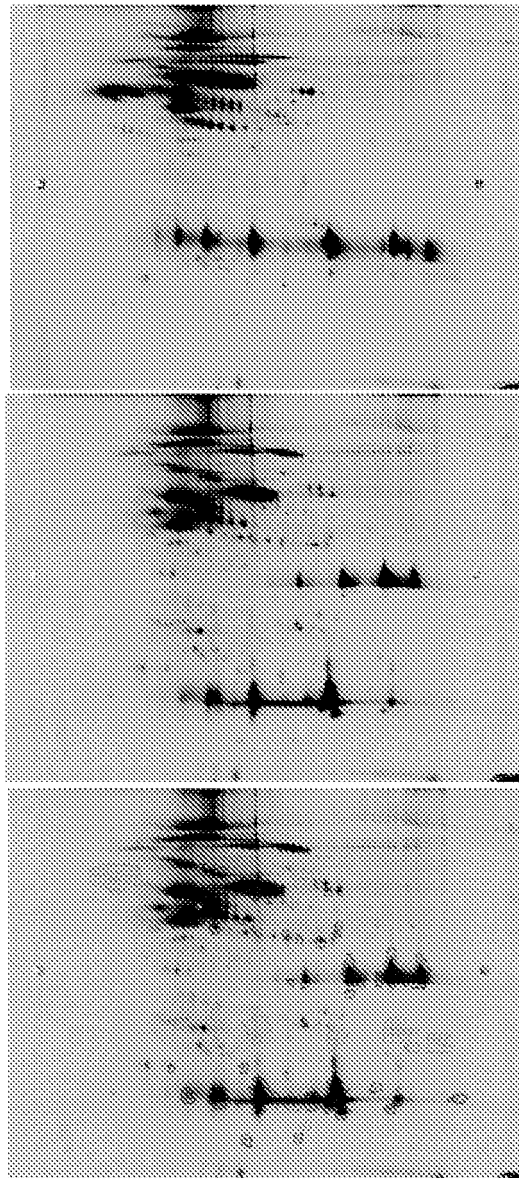


Figure 7

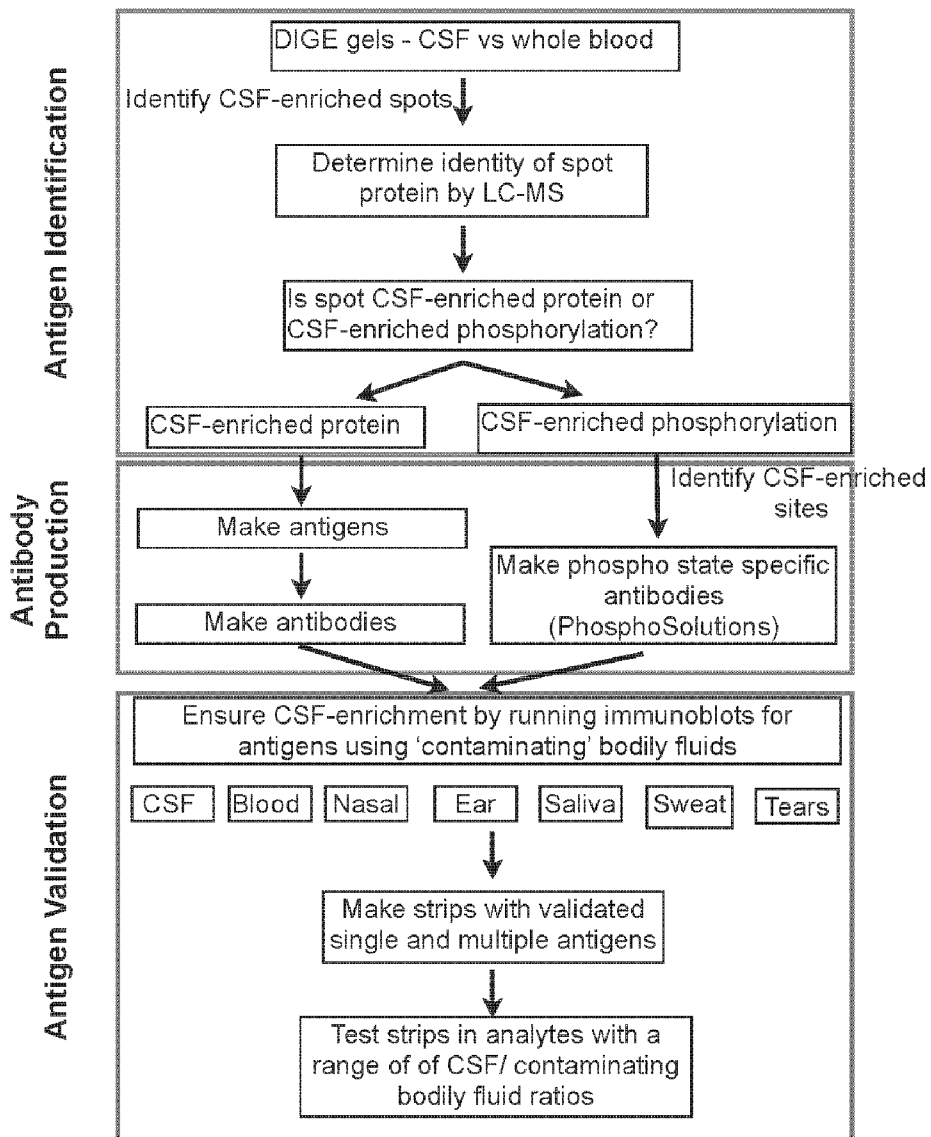


Figure 8

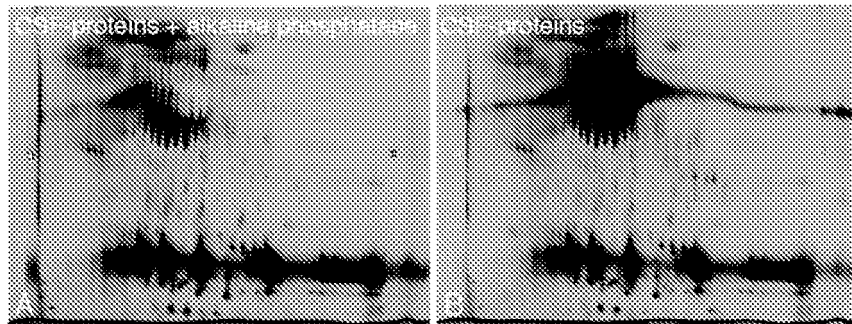


Figure 9

REFERENCES CITED IN THE DESCRIPTION

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摘要(译)

本公开涉及通过检测富含CSF的一种或多种抗原与其在其他体液中的水平相比检测样品中脑脊髓液 (CSF) 的存在或不存在。所述装置和方法适用于检测来自跨越种族，年龄，性别，健康状况和遗传变异性的各种人群的混合体液样品中脑脊液的存在或不存在。