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(71)申请人 欧蒙医学诊断技术有限公司
地址 德国吕贝克

(72)发明人 W·斯托克尔 B·特格 A·杨克

(74)专利代理机构 中国国际贸易促进委员会专利商标事务所 11038
代理人 张小勇

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(54)发明名称

诊断共孵育测定法

(57)摘要

本发明涉及一种诊断疾病的方法，所述方法包括使供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原的受体材料接触，然后检测包括所述抗体和所述抗原的复合物，以及包含供体组织切片和包含抗原的受体材料的诊断上有用的载体。

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1.一种诊断疾病的方法，包括：

使供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原的受体材料接触，

随后检测包含所述抗体和所述抗原的复合物。

2.根据权利要求1所述的方法，其中所述供体组织切片和所述包含抗原的受体材料在所述液体中共孵育。

3.一种诊断上有用的载体，其包含供体组织切片和包含抗原的受体材料，其中所述载体被构造成使得所述供体组织切片和所述包含抗原的受体材料可以在能够从所述供体组织切片提取抗体并将所述抗体转运到所述受体材料的液体中共孵育。

4.根据权利要求3所述的载体，其中所述供体组织切片和所述包含抗原的受体材料包被于所述载体的表面上，并且优选在空间上是分开的。

5.根据权利要求4所述的载体，其中所述载体包含具有用所述供体组织切片包被的表面的第一部分和具有用所述受体材料包被的表面的第二部分，

其中所述第一部分和第二部分是分开的，并且所述载体被构造成使得所述第一部分和第二部分可以接触，优选组装，使得所述第一部分或所述第二部分的表面面朝下地位于所述第一部分和所述第二部分中另一个的表面上，使得一滴液体可以置于第一部分和第二部分的表面之间，以允许任何来自所述供体组织切片的抗体扩散到所述受体材料。

6.根据权利要求4至5中任一项所述的载体，其中所述供体组织切片和所述包含抗原的受体材料在空间上分开的生物芯片上。

7.根据权利要求3至6中任一项所述的载体，其中能够从所述供体组织切片提取抗体的液体在所述载体的表面上，使得可以从供体组织切片提取抗体并且抗体通过所述液体扩散到所述包含抗原的受体材料。

8.根据权利要求1至7中任一项所述的方法或载体，其中已经从待诊断的患者获得所述供体组织切片。

9.根据权利要求1至8中任一项所述的方法或载体，其中所述包含抗原的受体材料是

包含优选为天然多肽形式的抗原的组织样品，

产生所述抗原的细胞，或

分离的多肽。

10.根据权利要求1至9中任一项所述的方法或诊断上有用的载体，其中所述供体组织切片为冷冻组织切片。

11.根据权利要求1至10中任一项所述的方法或载体，其中

所述疾病是胃肠疾病，优选为炎性或自身免疫性胃肠疾病，更优选乳糜泻，

所述供体组织切片是胃肠道组织，优选十二指肠组织，以及

所述抗原是组织转谷氨酰胺酶或脱酰氨麦醇溶蛋白或其变体，优选脱酰氨麦醇溶蛋白或其变体的寡聚物。

12.根据权利要求1至10中任一项所述的方法或载体，其中

疾病是天疱疮和/或类天疱疮，优选大疱性类天疱疮，

所述供体组织切片是患病的皮肤组织，以及

所述受体材料包含来自Dsg1、Dsg3、NC16A、BP180、BP230、LAMA3、层粘连蛋白332、β4整

联蛋白和VII型胶原及其变体的一种或多种抗原。

13.根据权利要求1至10中任一项所述的方法或载体，其中

疾病是肺出血-肾炎综合征或SLE，

所述供体组织切片是患病的肾组织，以及

所述包含抗原的受体材料是或来源于选自抗肾小球基底膜、dsDNS、人上皮细胞(HEp-2)、pLA2R和THSD7A或其变体的材料。

14.根据权利要求1至10中任一项所述的方法或载体，其中

所述疾病是克罗恩病，

所述供体组织切片是患病的肠组织，以及

所述包含抗原的受体材料选自健康胰腺组织、CUZD1和GP2及其变体，后两者优选为表达CUZD1和/或GP2或其变体的细胞的形式。

15.根据权利要求1至10中任一项所述的方法或载体，其中

所述疾病是格雷夫斯病和/或桥本病，

所述供体组织切片来源于甲状腺，以及

所述包含抗原的受体材料是或来源于健康的甲状腺组织。

16.根据权利要求1至10中任一项所述的方法或载体，其中

所述疾病是肌炎，

所述供体组织切片是肌肉或皮肤，优选肌肉组织，以及

所述包含抗原的受体材料是MUP-44或其变体。

诊断共孵育测定法

[0001] 本发明涉及一种诊断疾病的方法，所述方法包括使供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原的受体材料接触，然后检测包括所述抗体和所述抗原的复合物，以及包含供体组织切片和包含抗原的受体材料的诊断上有用的载体。

[0002] 在大多数人类中，默认地存在所有身体成分的耐受性，即它们不会触发针对其自身组织抗原的抗体的产生。相比之下，发生称为自身免疫疾病的病症，其中宿主对他自己的组织有明显的免疫反应，常常导致组织损伤。

[0003] 无论怎么强调早期可靠诊断自身免疫疾病的重要性都不过分。许多自身免疫疾病不能治愈，但可以使用可用于显著改善其症状的治疗，通常使患者能够正常生活。诊断越早，利用例如免疫抑制药物的施用或严格的无免疫原性抗原饮食来给患者提供全部益处的一系列疗法的机会越多。

[0004] 自身免疫疾病的诊断通常基于在包含抗体的液体样品中检测自身抗体的方法的结果(output)。最常使用从患者常规获得的血清。在其他情况下，使用如脑脊髓液(CSF)、特别是用于检测与神经症状相关的自身抗体的其它液体。

[0005] 乳糜泻(腹部疾病)(coeliac disease,CD)是自身免疫疾病的一个特例。它是由麸质(gIuten)引发的，其在遗传易感受试者中激活针对CD自身抗原(即组织转谷氨酰胺酶(TTG))的免疫反应。在发现针对麦醇溶蛋白和/或TTG的抗体的存在与CD相关联之后，可以确定CD不是罕见的，而是在全世界人群内预期患病率高于1％的常见病症。存在与麸质敏感性相关的一系列疾病，包括以摄取含麸质食物相关肠症状为特征的非腹腔麸质敏感性(Non Coeliac GIuten sensitivity,NCGS)，而在许多患者的情况下，仍然难以彻底区分它们。

[0006] 需要具有重要经验的病理学家来检查由活检样品制成的组织切片作为可靠诊断的基础。在许多情况下，这样的检查是不确定的，因为不能观察到特征性特征。然而，病理学家可能不具有由他处理的来自患者的血清样品以补充组织学分析，或者包含外周血的血清样品中抗体的浓度可能不足。

[0007] 在这种情况下，很可能会误诊患者。假阴性结果可能导致患者被拒绝适当治疗，导致继发性疾病的风险增加，其中包括例如肠淋巴瘤的癌症，以及生活质量差。

[0008] 因此，本发明内在的一个问题是提供一种诊断自身免疫疾病的方法，该方法可用于证实患病组织的不确定的组织学检查。另一个问题是提供具有增加的灵敏度并降低假阴性结果的可能性的改进的测试。

[0009] 本发明内在的至少一个问题在于提供一种可以在不存在血清样品的情况下进行的测定，并允许病理学家基于不确定的组织学检查证实诊断。

[0010] 本发明内在的问题由所附独立和从属权利要求的主题来解决。

[0011] 在第一方面，本发明内在的问题通过诊断疾病的方法来解决，该方法包括使供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原(任选两种或更多种抗原)的受体材料接触，随后检测包含所述抗体和所述抗原的复合物。

[0012] 在优选实施方案中，将所述供体组织切片和所述包含抗原的受体材料在所述液体

中共孵育。

[0013] 在第二方面，该问题通过包括供体组织切片和包含抗原的受体材料的诊断上有用的载体来解决，其中所述载体被构造成使得所述供体组织切片和所述包含抗原的受体材料可以在能够从所述供体组织切片提取抗体并将所述抗体转运到所述受体材料的液体中共孵育。

[0014] 在优选实施方案中，所述供体组织切片和所述包含抗原的受体材料包被于所述载体的表面上，并且优选在空间上是分开的。

[0015] 在优选实施方案中，供体组织切片和包含抗原的受体材料在空间上分开的生物芯片上。

[0016] 在优选实施方案中，所述载体被构造成使得能够从所述供体组织切片提取抗体的液体可以置于载体的表面上，使得可以从所述供体组织切片中提取抗体并且抗体通过所述液体扩散到所述包含抗原的受体材料。

[0017] 在优选实施方案中，所述载体包含具有用所述供体组织切片包被的表面的第一部分和具有用所述受体材料包被的表面的第二部分，

[0018] 其中所述第一部分和第二部分是分开的，并且所述载体被构造成使得所述第一部分和第二部分可以接触，优选组装，使得所述第一部分或所述第二部分的表面面朝下地位于所述第一部分和所述第二部分中另一个的表面上，使得一滴液体可以置于第一部分和第二部分的表面之间，以允许任何来自所述供体组织切片的抗体扩散到所述受体材料。

[0019] 在优选实施方案中，已经从待诊断的患者获得供体组织切片。

[0020] 在优选实施方案中，包含抗原的受体材料是包含优选天然多肽形式的抗原的组织样品，产生所述抗原的细胞或分离的多肽。

[0021] 在优选实施方案中，供体组织切片是冷冻组织切片。

[0022] 在优选实施方案中，疾病是胃肠疾病，优选炎性或自身免疫性胃肠疾病，更优选乳糜泻，供体组织切片是胃肠道组织，优选十二指肠组织，抗原是组织转谷氨酰胺酶或脱酰氨麦醇溶蛋白或其变体，优选脱酰氨麦醇溶蛋白或其变体的寡聚物。

[0023] 在一个优选实施方案中，该疾病是天疱疮和/或类天疱疮，优选大疱性类天疱疮，供体组织切片为患病的皮肤组织，受体材料包含来自Dsg1、Dsg3、NC16A、BP180、BP230、LAMA3(SEQ ID NO 14)，层粘连蛋白332的一种或多种抗原，其优选是包含包括LAMA3(SEQ ID NO 14)、LAMB3(SEQ ID NO 15)和LAMC2(SEQ ID NO 16)和β4整联蛋白(SEQ ID NO 17)和VII型胶原及其变体的多肽的组合物，任选以组织的形式，优选选自灵长类动物食管、人盐裂皮肤(human salt split skin)和大鼠膀胱。

[0024] 在优选实施方案中，所述疾病是肺出血-肾炎综合征或SLE，供体组织切片是患病的肾组织，并且包含抗原的受体材料是或来源于选自抗肾小球基底膜、dsDNS、人上皮细胞(HEp-2)、pLA2R和THSD7A及其变体的材料。

[0025] 在优选实施方案中，该疾病是克罗恩病，供体组织切片是患病的肠组织，并且包含抗原的受体材料选自健康胰腺组织、CUZD1和GP2及其变体，后两者优选为表达CUZD1和/或GP2或其变体的细胞的形式。

[0026] 在优选实施方案中，该疾病是格雷夫斯病和/或桥本病，供体组织切片来源于甲状腺，并且包含抗原的受体材料是或来源于健康的甲状腺组织。

[0027] 在优选实施方案中，疾病是肌炎，供体组织切片是肌肉或皮肤，优选肌肉组织，并且包含抗原的受体材料是MUP-44或其变体。

[0028] 本发明基于本发明人出乎意料的发现：可以在组织切片中检测自身抗体。例如，这适用于与CD相关的抗体。

[0029] 不希望受任何理论约束，本发明人假设自身抗体积累在病变组织和器官中，并且其转移到患者的血液是不完全的，不足以用于基于血清学的可靠分析。

[0030] 根据本发明，提供供体组织切片。这是从患有或怀疑患有待诊断的疾病的患者获得的一块组织。在优选实施方案中，将供体组织切片冷冻，优选浸入液氮或融化异戊烷。组织切片的厚度优选为1-100μm，优选为4-50μm。供体组织切片优选不用任何试剂处理或暴露于基本上改变供体组织切片中存在的抗体的条件。

[0031] 根据本发明，供体组织切片与能够从所述供体组织切片提取抗体的液体接触。这是任何液体，其在暴露于供体组织切片时可能接收供体组织切片中存在的抗体，保存其化学组成、结构或结合性质，例如特异性结合抗原的能力，并允许其递送到包含抗原的受体材料。优选地，液体是包含生理缓冲液的水性液体，更优选在pH 5至9，更优选6至8，最优选6.2至7.8。在最优选实施方案中，液体为PBS pH 7.4。

[0032] 在优选实施方案中，本文所用的术语“结合特异性”是指比以下特征结合反应更强的结合，其特征在于解离常数为1×10^{-5}M，更优选1×10^{-7}M，更优选1×10^{-8}M，更优选1×10^{-9}M，更优选1×10^{-10}M，更优选1×10^{-11}M，更优选1×10^{-12}M，如通过使用Biacore设备在25℃，pH 7.4的PBS缓冲液中的表面等离子体共振所测定的。

[0033] 能够提取抗体的液体优选包含有助于从组织中解离任何抗体同时保存其化学组成、结构或结合性质，特别是特异性结合抗原的能力的洗涤剂，并且优选为吐温。在优选实施方案中，液体包含0.1至10％，优选0.5至5％，更优选1至4％，最优选1.5至2.5％的洗涤剂。

[0034] 根据本发明，供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原的受体材料接触。受体材料包含允许抗体特异性结合的状态和构象的抗原。受体材料可以选自含有抗原的组织，优选天然多肽形式，更优选内源性天然多肽，产生抗原的细胞，优选过表达抗原的重组细胞和分离的多肽。细胞可以是完整的或裂解的细胞。

[0035] 在优选实施方案中，将供体组织切片和包含抗原的受体材料在液体中共孵育，即供体组织切片和受体材料二者都与液体接触，同时将抗体从供体组织切片提取并运送到受体材料。该共孵育步骤进行至少10、20、30、60分钟、2、3、4、6、8、10或12小时。

[0036] 或者，可以首先将供体组织切片与液体接触，然后分离供体组织切片和液体，并使液体与包含抗原的受体材料接触。在液体与包含抗原的受体材料接触之前，可以将液体储存、冷冻和/或浓缩。两个接触步骤可以进行至少10、20、30、60分钟、2、3、4、6、8、10或12小时。

[0037] 随后，将受体材料与能够提取抗体的液体和/或供体组织切片分离，例如通过分离载体的第一部分或第二部分，或通过例如通过抽吸方式除去液体。受体材料可以使用洗涤缓冲液洗涤至少一次。能够提取抗体的新鲜液体可以用作洗涤缓冲液，优选包含较小浓度的洗涤剂，例如在孵育步骤中使用的浓度的10％，或根本不含洗涤剂。

[0038] 随后，可以检测到包含抗体和抗原的任何复合物。在优选实施方案中，使用选自包括免疫扩散技术、免疫电泳技术、光散射免疫测定、凝集技术、标记的免疫测定法、例如包括放射性标记的免疫测定法、例如ELISA的免疫测定法、化学发光免疫测定法和免疫荧光技术。本领域技术人员熟悉这些方法。

[0039] 本发明涉及一种诊断上有用的载体，其优选是用于使供体组织切片和/或优选和包含抗原的受体材料与能够从供体组织切片提取抗体的液体接触的固体人造载体，所述供体组织切片和/或受体材料包被于所述载体表面上。优选地，供体组织切片和受体材料被疏水表面包围以固定能够提取抗体的液体的位置。在优选实施方案中，固体载体包含两个或多个分开的部分，一个包括供体组织切片，一个包含受体材料，每个分开的部分优选选自珠、微量滴定板、玻璃表面、生物芯片和膜，最优选生物芯片。

[0040] 在优选实施方案中，本文所用的术语“生物芯片”是指厚度为0.01至1mm，优选为0.02至0.5mm，更优选为0.05至0.4mm的平面薄载片。它们优选由玻璃或塑料制成。它们用生物制剂如供体组织切片或包含抗原的受体材料包被。

[0041] 在优选实施方案中，诊断上有用的载体可以用供体组织切片包被并包被有包含抗原的受体材料。供体组织切片和受体材料(优选二者均在生物芯片上)可以设定尺寸和可以非常接近地放置，使得能够提取抗体的一滴液体(优选体积为10至80μl，优选为20至60μl)可以同时与供体组织切片和受体材料接触以进行孵育步骤。

[0042] 优选地，在诊断上有用的载体上包被的供体组织切片和包被的包含抗原的受体材料在空间上分开，特别是使得可以独立于另一个从诊断上有用的载体添加或除去。

[0043] 在优选实施方案中，接触步骤可以如下进行：将一滴液体放置在表面上，并且使诊断载体与所述一滴液体接触，使得供体组织切片和受体材料(二者在诊断上有用的载体的同一侧上)在接触位于载体上的液滴时面朝下。由于其亲水性，表面、供体组织切片和受体材料粘附并固定液滴以用于整体的共孵育。抗体通过液体从供体组织切片向受体材料的提取和递送，可以通过轻轻摇动诊断上有用的载体与表面来得到支持。US2010/0124750中描述了用于进行孵育的装置。

[0044] 图1示出了实施根据本发明的方法的这种方式：诊断上相关的载体(1)，其包含包括抗体(3)的供体组织切片(2)和非常接近的包含抗原(5)的受体材料(4)，所述诊断上相关的载体(1)放置在亲水表面(6)上方，能够提取抗体(7)的一滴液体位于所述亲水表面(6)上。诊断相关的载体(1)放置在亲水表面(6)上方，使得抗体(3)可以通过液体从供体组织切片(2)递送到抗原(5)并形成复合物。随后，分离亲水表面和诊断上有用的载体。可以检测包含抗原和抗体的复合物(如果存在)。

[0045] 在另一个优选实施方案中，载体包括具有用所述供体组织切片包被的表面的第一部分和具有用所述包含抗原的受体材料包被的表面的第二部分，其中第一部分和第二部分是分开的并且载体被构造成使得第一部分和第二部分可以接触，优选组装，使得所述第一部分或所述第二部分的表面面朝下地位于所述第一部分和所述第二部分中另一个的表面上，使得一滴液体可以置于第一部分和第二部分的表面之间，以允许任何来自所述供体组织切片的抗体扩散到所述受体材料。图2示出了进行根据本发明的方法的这种方式。

[0046] 在另一个优选实施方案中，载体的第一部分是用包含抗原的受体材料包被的珠，并且与供体组织切片接触。后者优选包被于载体的第二部分的表面上，但也可以是非固定

的，例如漂浮在溶液中而不是包被。

[0047] 受体材料可以包括多肽抗原或由其组成，例如在组织、细胞中或以纯化形式由本申请中明确提及的确切序列表示，例如通过功能、名称、序列或登录号，或隐含提及，而且还包括这些多肽的变体。

[0048] 在优选实施方案中，本文所用的术语“变体”可以指所提到的全长序列的至少一个片段，更具体地相对于全长序列，在一个或两个末端被截短了一个或多个氨基酸的一个或多个氨基酸。这样的片段包含或编码具有原始序列或其变体的肽的至少6、7、8、10、12、15、20、25、50、75、100、150或200个连续氨基酸。变体的总长度可以是至少6、7、8、9、10、11、12、20、25、30、40、50、60、70、80、90、100个或更多个氨基酸。变体可以包括与提到的参考氨基酸序列或所述参考氨基酸序列的相应片段至少40、50、60、70、75、80、85、90、92、94、95、96、97、98或99％相同的全长序列或片段。

[0049] 此外，其任何变体还可包含化学修饰，例如同位素标记或共价修饰，例如糖基化、磷酸化、乙酰化、脱羧、瓜氨酸化、甲基化、羟基化等。

[0050] 优选包含抗原的多肽的变体具有生物活性。在优选实施方案中，这样的生物学活性是特异性结合抗体的能力，优选在患有相应疾病的患者中发现的感兴趣的自身抗体。例如，抗原如脱酰氨麦醇溶蛋白的变体的生物活性可以是特异性结合患者抗体的脱酰氨麦醇溶蛋白的能力，其存在表明患者患有疾病，例如在脱酰氨麦醇溶蛋白变体的情况下患者患有CD。

[0051] 多肽或其变体可以以任何形式和以任何纯化的程度来提供，包括，以内源形式包含所述多肽的液体样品、组织或细胞(更优选过表达多肽的细胞，此类细胞的粗产物或富集裂解物)，以及包括纯化和/或分离的多肽，其任选地基本上是纯的。在优选实施方案中，多肽是天然多肽，其中如本文所用的术语“天然多肽”是指折叠的多肽，更优选从组织或细胞、更优选哺乳动物细胞或组织、任选地来自非重组组织或细胞纯化的折叠多肽。在另一个优选实施方案中，多肽是重组蛋白，其中如本文所用的术语“重组蛋白”是指在生产过程的任何阶段使用遗传工程方法产生的多肽。在优选实施方案中，如果通过SDS PAGE然后进行考马斯蓝染色和目视检查判断的相应样品中至少60、70、80、90、95或99％的多肽由所述多肽组成，则多肽是纯的。重组多肽可以包含两个或更多个抗原拷贝，优选彼此融合。

[0052] 如果以组织形式提供抗原，则组织优选为哺乳动物组织，例如人、大鼠、灵长类动物、驴、小鼠、山羊、马、绵羊、猪或牛。

[0053] 根据本发明，提供了一种诊断疾病的方法。在优选实施方案中，本文所用的术语“诊断”是指旨在获得有用信息的任何种类的方法，所述信息有助于评估患者是否患有或可能或更可能比普通受试者或比较受试者(后者优选具有相似的症状)在过去、在诊断时或在将来患有某种疾病或病症，以了解疾病进展情况或将来可能进展或评估患者对某种治疗例如免疫抑制药物的施用的应答情况。换句话说，术语“诊断”不仅包括疾病或病症的诊断，而且包括预测和/或监测疾病或病症的过程。

[0054] 在许多情况下，仅仅检测，换句话说，确定样品中是否存在可检测的抗体水平，对于诊断是有用的，因为它表明患者患有疾病的可能性增加。在优选实施方案中，可以测定血清中抗体相对于普通健康受试者中可能发现的水平的相对浓度。尽管在许多情况下，测定样品中自身抗体是否存在或可检测可能是足够的，但为了获得诊断有用的信息而进行的方

法可以包括测定浓度是否是比普通健康受试者中发现浓度高至少0.1、优选0.2、0.5、1、2、5、10、20、25、50、100、200、500、1000、10000或100000倍。

[0055] 本领域技术人员将理解，临床医师通常不会仅仅基于单个诊断参数而断定患者是否患有或可能患有疾病、病况或病症，而是还基于其他方面，例如其他自身抗体、标志物的存在、血液参数、患者症状的临床评估或医学成像或其他非侵入性方法如多导睡眠监测仪的结果，以得出确定的诊断。(参见BaenkIer H.W.(2012),GeneraI aspects of autoimmune diagnostics,in Renz,H.,Autoimmune diagnostics,2012,de Gruyter,第3页)。诊断剂或方法的价值也可能是属于这样的可能性，排除一种疾病，从而允许间接诊断另一种疾病。

[0056] 术语“诊断”还可以指用于区分与相似或相同症状相关联的两种或多种病况的方法或药剂。术语“诊断”也可以指用于选择对于患者最有希望的治疗方案的方法或药剂。换句话说，该方法或药剂可涉及选择受试者的治疗方案。

[0057] 在优选实施方案中，待检测的抗体是自身抗体。在另一个优选实施方案中，待检测的抗体是IgG或IgA类抗体，优选IgA类抗体。

[0058] 证实存在或不存在包含抗体和本发明多肽的复合物的任何数据可以与参考数据相关联。例如，所述复合物的检测表示提供所分析的样本的患者已经患有、正在患有或者可能在将来遭受疾病。如果先前已经诊断出患者并且再次运行获得诊断相关信息的方法，则在两次运行中检测到的复合物的量可能相关，以了解疾病的进展和/或治疗的成功。例如，如果发现复合物的量增加，这表明该疾病正在进展，可能在未来显现和/或表明任何尝试的治疗是不成功的。

[0059] 本发明的教导提供了一种试剂盒，优选用于诊断疾病的试剂盒，其包含根据本发明的诊断上有用的载体。诊断上有用的载体可以最初包含仅包含抗原的受体材料，并且被构造成使得最终用户可以在实施本发明的方法之前添加供体组织切片。另外，所述试剂盒可以包括详细说明如何使用试剂盒的说明书和用于使本发明多肽与来自受试者、优选人受试者的体液样品接触的手段。此外，试剂盒可以包含阳性对照(例如已知结合本发明多肽的一批抗体或重组抗体)和阴性对照(例如对本发明多肽无可检测亲和力的蛋白质如牛血清白蛋白)。最后，这样的试剂盒可以包含用于制备校准曲线的抗体或抗原的标准溶液。

[0060] 在优选实施方案中，试剂盒包括用于检测与在受体材料中抗原结合的抗体的手段，优选通过检测包含本发明多肽和与本发明多肽结合的抗体的复合物。此类手段优选是结合所述复合物并修饰复合物或携带使得复合物可检测到的标记的试剂。例如，所述手段可以是在除了由第一抗体识别的结合位点结合所述多肽或结合第一抗体的恒定区的标记抗体。或者，所述手段可以是与抗体的恒定区结合的第二抗体，优选特异性针对人抗体的第二抗体。

[0061] 在优选实施方案中，待诊断的疾病是胃肠疾病，优选炎性或自身免疫性胃肠疾病，更优选乳糜泻，供体组织切片是胃肠道组织，优选十二指肠组织，并且抗原选自组织转谷氨酰胺酶(Uniprot数据库:P21980,参考优先权数据在线版本，作为本文引用的所有数据库编号)或其变体或选自包含SEQ ID NO 1、SEQ ID NO 3、SEQ ID NO 4、SEQ ID NO5及其变体的麦醇溶蛋白变体，优选其融合的多于一个拷贝，例如SEQ ID NO 2。方法或载体可用于诊断NCGS，优选区分与麸质敏感性相关的胃肠病，更优选CD和NCGS。待检测的抗体优选为TTG或

脱酰氨麦醇溶蛋白、更优选为脱酰氨麦醇溶蛋白的IgA类抗体。

[0062] 在另一个优选实施方案中，待诊断的疾病是天疱疮和/或类天疱疮，优选大疱性类天疱疮，供体组织切片为患病皮肤组织，抗原选自Dsg1(Uniprot数据库编号Q02413)Dsg3(Uniprot数据库编号P32926)、NC16A(SEQ ID NO 6)，任选以包含SEQ ID NO 7的两个或更多个拷贝的融合体的形式、BP180(Uniprot数据库编号Q9UMD9)、BP 230(SEQ ID NO 8)和VII型胶原(Uniprot数据库编号Q02388)或其变体。

[0063] 在另一个优选实施方案中，待诊断的疾病是肺出血-肾炎综合征或SLE，供体组织切片是患病的肾组织，并且包含抗原的受体材料选自抗肾小球基底膜、dsDNS、人上皮细胞(HEp-2)、pLA2R(SEQ ID NO 9)和THSD7A(SEQ ID NO 10)或其变体。

[0064] 在另一个优选实施方案中，待诊断的疾病是克罗恩病，供体组织切片是患病的肠组织，抗原选自CUZD1(SEQ ID NO 11)和GP2(SEQ ID NO 12)或其变体。

[0065] 在另一个优选实施方案中，待诊断的疾病是格雷夫斯病和/或桥本病，供体组织切片来源于甲状腺，并且抗原是或来源于健康的甲状腺组织。

[0066] 在另一个优选实施方案中，待诊断的疾病是肌炎，供体组织切片是肌肉或皮肤，优选肌肉组织，并且抗原是或来源于MUP-44(SEQ ID NO 13)或其变体。

[0067] 通过非限制性附加附图和以下非限制性示例进一步说明本发明，从中可以得到本发明的其它特征、实施方案、方面和优点。

[0068] 图3示出了本发明中使用的包含BIOCHIP马赛克的载片。

[0069] 图4显示了皮肤活检样品以及各种受体材料的位置，更具体地分别显示表达桥粒黏蛋白1、BP230gc(SEQ ID NO 18)、桥粒黏蛋白3和BP180-NC16A的HEK293细胞。

[0070] 图5显示了阳性活检样品的免疫荧光分析，其中可检测到BP230的自身抗体。

[0071] 图6显示了阳性活检样品的免疫荧光分析，其中可检测到桥粒黏蛋白3的自身抗体。

[0072] 实施例1：

[0073] 制造根据本发明的诊断上有用的载体及其用途

[0074] 在显微镜载片上的一个反应场内将用作为组织供体切片的活检样品(冷冻于液氮中)的冷冻切片(4μm)包被的生物芯片放置在用作为包含抗原的受体材料的纯化的重组抗原GAF-3X(EUROPLUS)(SEQ ID NO 2)的点包被的生物芯片旁边。从其他患者获得的所有活检样品都重复了这一步骤。

[0075] 在4℃下将包含两个生物芯片的显微镜载片和一滴与活检样品和抗原二者放置接触的PBST缓冲液(含有2％(v/v)吐温-20的PBS，pH 7.4)孵育过夜。这样，从组织洗脱的抗体可以扩散到缓冲液中，结合脱酰氨麦醇溶蛋白的抗体可以结合相邻的GAF-3X抗原点并被检测到。第二天，使用PBST(含有0.2％(v/v)吐温-20的PBS，pH 7.4)洗涤生物芯片5分钟。随后，将生物芯片与FITC缀合的抗人IgA抗体(Euroimmun AG)孵育30分钟，并再次洗涤5分钟。最后，使用荧光显微镜(EUROStar)分析载片。

[0076] 样品

[0077] 经分析的队列包括37名患有乳糜泻的患者和35名健康对照人员，所述患者经根据有经验的临床医师对活检样品进行组织学检查而得到诊断。获得来自十二指肠球部(BuIbus duodeni)的十二指肠活检样品(36名CD患者，34名对照个体)。所有个体的血清样

品可供使用。将样品于暗处(通过盲式)孵育和显微镜分析,并对数据进行最终评估。

[0078] 使用市售抗GAF-3X-IIFT(IgA)和抗GAF-3X ELISA(IgA)根据制造商的说明书(EUROIMMUN AG,Germany,产品号分别是FV 3011-#A和EV 3011-9601A)另外测试血清样品。

[0079] 81份血清样品的血清学分析(不是根据本发明)

[0080] 使用免疫荧光试验,37个测试CD患者中28个样品为IgA抗GAF-3X抗体阳性,灵敏度为76%。在35个对照样品中,抗GAF-3X-IIFT(IgA)中没有阳性。因此,IIFT(IgA)达到100%的特异性。

[0081] 使用根据本发明的方法获得的IgA检测的增强的灵敏度

[0082] 在根据本发明的方法中使用十二指肠球部(BuIbus duodeni)活检样品,36名CD患者(其样品可获得)中有32名对于IgA抗GAF-3X抗体检测为阳性。在32名患者中,5名在ELISA和IIFT二者中均检测为阴性。进一步测试对照个体的34例十二指肠球部(buIbus)活检样品,其中仅一例显示IgA类抗GAF-3X抗体。

[0083] 总之,对于IgA抗GAF-3X,十二指肠球部(BuIbus duodeni)活检样品共孵育测试灵敏度为89%,从而达到97%的特异性。

[0084] 结果表明,与脱酰氨麦醇溶蛋白结合的抗体存在于小肠粘膜内,并且使用本发明的方法在来自患者血清样品为阴性的组织切片中可以检测到。结果,可减少假阴性结果的数量。

[0085] 实施例2:

[0086] 大疱性类天疱疮(BP)和寻常型天疱疮(PV)与针对BP180、BP230和桥粒黏蛋白(Dsg1、Dsg3)的循环自身抗体相关。

[0087] 针对BP 180和/或BP230的抗体产生大疱性类天疱疮的血清学指征。它也可以是很少发现的扁平苔藓类天疱疮或类似的不常见的粘膜类天疱疮(仅BP180)或瘢痕性类天疱疮(仅BP180),其主要发生在老年人中。在孕妇中,应考虑妊娠类天疱疮。

[0088] 抗桥粒黏蛋白1的抗体指示落叶型天疱疮病,而抗桥粒黏蛋白3的抗体(有时另外还包括抗桥粒黏蛋白1的抗体)出现在寻常型天疱疮中。

[0089] 活检样品的直接免疫荧光(DIF)显示天疱疮病中的桥粒和类天疱疮病中的表皮基底膜的染色。为此,制备患者活检材料的组织切片,用荧光染料标记的抗人单克隆抗体孵育,然后使用荧光显微镜进行评价。

[0090] 然后,血清学鉴别必须用单特异性测试(例如通过与重组HEK细胞(表达特异性抗原)的间接免疫荧光(IIF))和抗原制剂(EUROPLUS®)进行。这一步对于确定诊断很重要,因为几种靶抗原分别适合于基底膜和桥粒的荧光。BIOCHIPTM-马赛克(由在一个反应场内若干用组织、细胞底物或小抗原点的制剂(EUROPLUS®)包被的小玻璃芯片组成)与患者血清和荧光标记的抗血清连续孵育。然后根据制造商的说明进行显微评估。

[0091] 本研究的目的是通过活检样品和重组蛋白的细胞制剂或抗原点在IIF(与使用血清样品进行的常规IIFT相反)中的共孵育单特异性测定从组织洗脱的抗体。这样就可以一步进行搜索测试(通常是DIF)和确认测试(通常是IIFT)。

[0092] 将一例PV和两例BP患者的活检样品的冷冻切片分别在具有用桥粒黏蛋白1(EUROIMMUN,底物FD 1495-50;DSG1)、桥粒黏蛋白3(EUROIMMUN,底物FD 1496-50;DSG3)、BP230(EUROIMMUN底物FD 1502-56;BP230gC(SEQ ID NO 18))或抗BP180-NC16A-4X

(EUROIMMUN底物FD 1502-52;BP180-NC16A-4X)转染的HEK293细胞的一个载片上组合成BIOCHIP™-马赛克(图1和2)。将组织切片、丙酮或甲醛固定的重组细胞制剂或抗原制剂(EUROPLUS®)用作抗原底物。使用EP专利0 117 262中描述的技术。简言之,将用自发反应性醛基化学包被的非常薄的玻璃板用抗原覆盖。细胞培养物直接在玻璃板上生长。组织切片的游离氨基,特别是胶原蛋白的羟赖氨酸共价粘附到载体材料上,使得组织不能通过用液体试剂进行以下孵育而被去除。玻璃板工业上切割成具有1-2mm边缘长度的非常小的玻璃芯片。用不同抗原包被的玻璃芯片可以在载片的一个反应场中组合以获得所谓的BIOCHIP-Mosaic™。

[0093] 将个体马赛克与30μl PBS-吐温20(2%)在4℃孵育15小时。通过FITC标记的抗人IgG抗体(EUROIMMUN,产品号AF 102,FITC标记的抗人IgG(山羊))显现结合的抗体。室温(20-25℃)孵育30分钟。两个孵育步骤后将载片用PBS-吐温20(0.2%)洗涤5分钟。然后覆盖它们并在荧光显微镜(Axio Scope A1,Zeiss,Jena,Germany,第490035-9100-000号)下进行评估。

[0094] PV活检样品的共孵育显示与桥粒和Dsg3的平行反应性(参见图6的示例性图像),而两例BP活检样品的共孵育导致表皮基底膜和BP180和BP230抗原的染色(参见图5的示例性图像)。与在患者血清中检测到抗体的常规IIFT相反,使用共孵育技术检测到存在于活检样品内体液残留物中的抗体。

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20 25 30

GIn Leu Pro GIn Phe GIu GIu Met Pro Leu GIn Pro GIu GIn Pro Phe
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Pro GIu GIn Leu Pro GIn Phe GIu GIu Met VaI His His His His His
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His
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Asp Arg Leu GIn GIy Met AIa Pro AIa AIa GIy AIa Asp Leu Asp Lys
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IIe GIy Leu His Ser Asp Ser GIn GIu GIu Leu Trp Met Phe VaI Arg
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Lys Lys Leu Met Met GIu GIn GIu Asn
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35 40 45
GIy Met AIa Pro AIa AIa GIy AIa Asp Leu Asp Lys IIe GIy Leu His
50 55 60
Ser Asp Ser GIn GIu GIu Leu Trp Met Phe VaI Arg Lys Lys Leu Met
65 70 75 80
Met GIu GIn GIu Asn GIy Thr GIu GIu VaI Arg Lys Leu Lys AIa Arg
85 90 95
VaI Asp GIu Leu GIu Arg IIe Arg Arg Ser IIe Leu Pro Tyr GIy Asp
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Ser Met Asp Arg IIe GIu Lys Asp Arg Leu GIn GIy Met AIa Pro AIa
115 120 125
AIa GIy AIa Asp Leu Asp Lys IIe GIy Leu His Ser Asp Ser GIn GIu
130 135 140
GIu Leu Trp Met Phe VaI Arg Lys Lys Leu Met Met GIu GIn GIu Asn
145 150 155 160
Lys Leu GIu GIu VaI Arg Lys Leu Lys AIa Arg VaI Asp GIu Leu GIu
165 170 175
Arg IIe Arg Arg Ser IIe Leu Pro Tyr GIy Asp Ser Met Asp Arg IIe
180 185 190
GIu Lys Asp Arg Leu GIn GIy Met AIa Pro AIa AIa GIy AIa Asp Leu
195 200 205
Asp Lys IIe GIy Leu His Ser Asp Ser GIn GIu GIu Leu Trp Met Phe
210 215 220

Val Arg Lys Lys Leu Met Met Glu Gln Glu Asn Gly Ser Glu Glu Val
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Arg Lys Leu Lys Ala Arg Val Asp Glu Leu Glu Arg Ile Arg Arg Ser
245 250 255
Ile Leu Pro Tyr Gly Asp Ser Met Asp Arg Ile Glu Lys Asp Arg Leu
260 265 270
Gln Gly Met Ala Pro Ala Ala Gly Ala Asp Leu Asp Lys Ile Gly Leu
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35 40 45
Glu Glu Lys Trp Gln His Arg Val Val Glu Gln Ile Pro Lys Glu Val
50 55 60
Gln Phe Gln Pro Pro Gly Ala Pro Leu Glu Lys Glu Lys Ser Gln Gln
65 70 75 80
Cys Tyr Ser Glu Tyr Phe Ser Gln Thr Ser Thr Glu Leu Gln Ile Thr
85 90 95
Phe Asp Glu Thr Asn Pro Ile Thr Arg Leu Ser Glu Ile Glu Lys Ile
100 105 110
Arg Asp Gln Ala Leu Asn Asn Ser Arg Pro Pro Val Arg Tyr Gln Asp
115 120 125
Asn Ala Cys Glu Met Glu Leu Val Lys Val Leu Thr Pro Leu Glu Ile
130 135 140
Ala Lys Asn Lys Gln Tyr Asp Met His Thr Glu Val Thr Thr Leu Lys
145 150 155 160

Gln Glu Lys Asn Pro Val Pro Ser Ala Glu Glu Trp Met Leu Glu Gly
165 170 175
Cys Arg Ala Ser Gly Gly Leu Lys Lys Gly Asp Phe Leu Lys Lys Gly
180 185 190
Leu Glu Pro Glu Thr Phe Gln Asn Phe Asp Gly Asp His Ala Cys Ser
195 200 205
Val Arg Asp Asp Glu Phe Lys Phe Gln Gly Leu Arg His Thr Val Thr
210 215 220
Ala Arg Gln Leu Val Glu Ala Lys Leu Leu Asp Met Arg Thr Ile Glu
225 230 235 240
Gln Leu Arg Leu Gly Leu Lys Thr Val Glu Glu Val Gln Lys Thr Leu
245 250 255
Asn Lys Phe Leu Thr Lys Ala Thr Ser Ile Ala Gly Leu Tyr Leu Glu
260 265 270
Ser Thr Lys Glu Lys Ile Ser Phe Ala Ser Ala Ala Glu Arg Ile Ile
275 280 285
Ile Asp Lys Met Val Ala Leu Ala Phe Leu Glu Ala Gln Ala Ala Thr
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Gly Phe Ile Ile Asp Pro Ile Ser Gly Gln Thr Tyr Ser Val Glu Asp
305 310 315 320
Ala Val Leu Lys Gly Val Val Asp Pro Glu Phe Arg Ile Arg Leu Leu
325 330 335
Glu Ala Glu Lys Ala Ala Val Gly Tyr Ser Tyr Ser Ser Lys Thr Leu
340 345 350
Ser Val Phe Gln Ala Met Glu Asn Arg Met Leu Asp Arg Gln Lys Gly
355 360 365
Lys His Ile Leu Glu Ala Gln Ile Ala Ser Gly Gly Val Ile Asp Pro
370 375 380
Val Arg Gly Ile Arg Val Pro Pro Glu Ile Ala Leu Gln Gln Gly Leu
385 390 395 400
Leu Asn Asn Ala Ile Leu Gln Phe Leu His Glu Pro Ser Ser Asn Thr
405 410 415
Arg Val Phe Pro Asn Pro Asn Asn Lys Gln Ala Leu Tyr Tyr Ser Glu
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Leu Leu Arg Met Cys Val Phe Asp Val Glu Ser Gln Cys Phe Leu Phe
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Pro Phe Gly Glu Arg Asn Ile Ser Asn Leu Asn Val Lys Lys Thr His
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Arg Ile Ser Val Val Asp Thr Lys Thr Gly Ser Glu Leu Thr Val Tyr

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Gly His Ser Ser His Met Leu Thr Asp Thr Lys Thr Gly Leu His Phe
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Lys Lys Tyr Gln Glu Gly Leu Ile Thr Leu Thr Glu Leu Ala Asp Ser
545 550 555 560
Leu Leu Ser Arg Leu Val Pro Lys Lys Asp Leu His Ser Pro Val Ala
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Gly Tyr Trp Leu Thr Ala Ser Gly Glu Arg Ile Ser Val Leu Lys Ala
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Ala Gln Val Ser Thr Gly Gly Ile Ile Asp Pro Leu Thr Gly Lys Lys
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Tyr Arg Val Ala Glu Ala Leu His Arg Gly Leu Val Asp Glu Gly Phe
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Ala Gln Gln Leu Arg Gln Cys Glu Leu Val Ile Thr Gly Ile Gly His
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Pro Ile Thr Asn Lys Met Met Ser Val Val Glu Ala Val Asn Ala Asn
660 665 670
Ile Ile Asn Lys Glu Met Gly Ile Arg Cys Leu Glu Phe Gln Tyr Leu
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690 695 700
Glu Ala Leu Gln Val Gly Ile Ile Asp Val Leu Ile Ala Thr Lys Leu
705 710 715 720
Lys Asp Gln Lys Ser Tyr Val Arg Asn Ile Ile Cys Pro Gln Thr Lys
725 730 735
Arg Lys Leu Thr Tyr Lys Glu Ala Leu Glu Lys Ala Asp Phe Asp Phe
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35 40 45
Lys Lys Cys Ile Gln Ala Gly Lys Ser Val Leu Thr Leu Glu Asn Cys
50 55 60
Lys Gln Ala Asn Lys His Met Leu Trp Lys Trp Val Ser Asn His Gly
65 70 75 80
Leu Phe Asn Ile Gly Gly Ser Gly Cys Leu Gly Leu Asn Phe Ser Ala
85 90 95
Pro Glu Gln Pro Leu Ser Leu Tyr Glu Cys Asp Ser Thr Leu Val Ser
100 105 110
Leu Arg Trp Arg Cys Asn Arg Lys Met Ile Thr Gly Pro Leu Gln Tyr
115 120 125
Ser Val Gln Val Ala His Asp Asn Thr Val Val Ala Ser Arg Lys Tyr
130 135 140
Ile His Lys Trp Ile Ser Tyr Gly Ser Gly Gly Gly Asp Ile Cys Glu
145 150 155 160
Tyr Leu His Lys Asp Leu His Thr Ile Lys Gly Asn Thr His Gly Met
165 170 175
Pro Cys Met Phe Pro Phe Gln Tyr Asn His Gln Trp His His Glu Cys
180 185 190
Thr Arg Glu Gly Arg Glu Asp Asp Leu Leu Trp Cys Ala Thr Thr Ser
195 200 205
Arg Tyr Glu Arg Asp Glu Lys Trp Gly Phe Cys Pro Asp Pro Thr Ser
210 215 220
Ala Glu Val Gly Cys Asp Thr Ile Trp Glu Lys Asp Leu Asn Ser His
225 230 235 240
Ile Cys Tyr Gln Phe Asn Leu Leu Ser Ser Leu Ser Trp Ser Glu Ala
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His Ser Ser Cys Gln Met Gln Gly Gly Thr Leu Leu Ser Ile Thr Asp
260 265 270
Glu Thr Glu Glu Asn Phe Ile Arg Glu His Met Ser Ser Lys Thr Val
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Glu Val Trp Met Gly Leu Asn Gln Leu Asp Glu His Ala Gly Trp Gln
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Trp Ser Asp Gly Thr Pro Leu Asn Tyr Leu Asn Trp Ser Pro Glu Val
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325 330 335
Met Pro Ser Ala Trp Arg Ser Arg Asp Cys Glu Ser Thr Leu Pro Tyr
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Ile Cys Lys Lys Tyr Leu Asn His Ile Asp His Glu Ile Val Glu Lys
355 360 365
Asp Ala Trp Lys Tyr Tyr Ala Thr His Cys Glu Pro Gly Trp Asn Pro
370 375 380
Tyr Asn Arg Asn Cys Tyr Lys Leu Gln Lys Glu Glu Lys Thr Trp His
385 390 395 400
Glu Ala Leu Arg Ser Cys Gln Ala Asp Asn Ser Ala Leu Ile Asp Ile
405 410 415
Thr Ser Leu Ala Glu Val Glu Phe Leu Val Thr Leu Leu Gly Asp Glu
420 425 430
Asn Ala Ser Glu Thr Trp Ile Gly Leu Ser Ser Asn Lys Ile Pro Val
435 440 445
Ser Phe Glu Trp Ser Asn Asp Ser Ser Val Ile Phe Thr Asn Trp His
450 455 460
Thr Leu Glu Pro His Ile Phe Pro Asn Arg Ser Gln Leu Cys Val Ser
465 470 475 480
Ala Glu Gln Ser Glu Gly His Trp Lys Val Lys Asn Cys Glu Glu Arg
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Leu Phe Tyr Ile Cys Lys Lys Ala Gly His Val Leu Ser Asp Ala Glu
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Ser Gly Cys Gln Glu Gly Trp Glu Arg His Gly Gly Phe Cys Tyr Lys
515 520 525
Ile Asp Thr Val Leu Arg Ser Phe Asp Gln Ala Ser Ser Gly Tyr Tyr
530 535 540
Cys Pro Pro Ala Leu Val Thr Ile Thr Asn Arg Phe Glu Gln Ala Phe
545 550 555 560
Ile Thr Ser Leu Ile Ser Ser Val Val Lys Met Lys Asp Ser Tyr Phe

565 570 575

Trp Ile Ala Leu Gln Asp Gln Asn Asp Thr Gly Glu Tyr Thr Trp Lys

580 585 590

Pro Val Gly Gln Lys Pro Glu Pro Val Gln Tyr Thr His Trp Asn Thr

595 600 605

His Gln Pro Arg Tyr Ser Gly Gly Cys Val Ala Met Arg Gly Arg His

610 615 620

Pro Leu Gly Arg Trp Glu Val Lys His Cys Arg His Phe Lys Ala Met

625 630 635 640

Ser Leu Cys Lys Gln Pro Val Glu Asn Gln Glu Lys Ala Glu Tyr Glu

645 650 655

Glu Arg Trp Pro Phe His Pro Cys Tyr Leu Asp Trp Glu Ser Glu Pro

660 665 670

Gly Leu Ala Ser Cys Phe Lys Val Phe His Ser Glu Lys Val Leu Met

675 680 685

Lys Arg Thr Trp Arg Glu Ala Glu Ala Phe Cys Glu Glu Phe Gly Ala

690 695 700

His Leu Ala Ser Phe Ala His Ile Glu Glu Glu Asn Phe Val Asn Glu

705 710 715 720

Leu Leu His Ser Lys Phe Asn Trp Thr Glu Glu Arg Gln Phe Trp Ile

725 730 735

Gly Phe Asn Lys Arg Asn Pro Leu Asn Ala Gly Ser Trp Glu Trp Ser

740 745 750

Asp Arg Thr Pro Val Val Ser Ser Phe Leu Asp Asn Thr Tyr Phe Gly

755 760 765

Glu Asp Ala Arg Asn Cys Ala Val Tyr Lys Ala Asn Lys Thr Leu Leu

770 775 780

Pro Leu His Cys Gly Ser Lys Arg Glu Trp Ile Cys Lys Ile Pro Arg

785 790 795 800

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Leu Phe Tyr Gln Asp Ala Glu Tyr Leu Phe His Thr Phe Ala Ser Glu

820 825 830

Trp Leu Asn Phe Glu Phe Val Cys Ser Trp Leu His Ser Asp Leu Leu

835 840 845

Thr Ile His Ser Ala His Glu Gln Glu Phe Ile His Ser Lys Ile Lys

850 855 860

Ala Leu Ser Lys Tyr Gly Ala Ser Trp Trp Ile Gly Leu Gln Glu Glu

865 870 875 880

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Arg Cys Gly Phe Ile Ser Ser Ile Thr Gly Leu Trp Gly Ser Glu Glu
915 920 925
Cys Ser Val Ser Met Pro Ser Ile Cys Lys Arg Lys Lys Val Trp Leu
930 935 940
Ile Glu Lys Lys Lys Asp Thr Pro Lys Gln His Gly Thr Cys Pro Lys
945 950 955 960
Gly Trp Leu Tyr Phe Asn Tyr Lys Cys Leu Leu Leu Asn Ile Pro Lys
965 970 975
Asp Pro Ser Ser Trp Lys Asn Trp Thr His Ala Gln His Phe Cys Ala
980 985 990
Glu Glu Gly Gly Thr Leu Val Ala Ile Glu Ser Glu Val Glu Gln Ala
995 1000 1005
Phe Ile Thr Met Asn Leu Phe Gly Gln Thr Thr Ser Val Trp Ile Gly
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Tyr Ser Asn Trp Ser Pro Phe Asp Ile Ile Asn Ile Pro Ser His Asn
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Lys Glu Gly Tyr Gly Phe Val Cys Glu Lys Met Gln Asp Thr Ser Gly
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His Gly Val Asn Thr Ser Asp Met Tyr Pro Met Pro Asn Thr Leu Glu
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Tyr Gly Asn Arg Thr Tyr Lys Ile Ile Asn Ala Asn Met Thr Trp Tyr
1125 1130 1135
Ala Ala Ile Lys Thr Cys Leu Met His Lys Ala Gln Leu Val Ser Ile
1140 1145 1150
Thr Asp Gln Tyr His Gln Ser Phe Leu Thr Val Val Leu Asn Arg Leu
1155 1160 1165
Gly Tyr Ala His Trp Ile Gly Leu Phe Thr Thr Asp Asn Gly Leu Asn
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Phe Asp Trp Ser Asp Gly Thr Lys Ser Ser Phe Thr Phe Trp Lys Asp

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Arg Trp His Ser Thr Ala Cys Glu Ser Phe Leu Gln Gly Ala Ile Cys
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His Val Pro Pro Glu Thr Arg Gln Ser Glu His Pro Glu Leu Cys Ser
1235 1240 1245
Glu Thr Ser Ile Pro Trp Ile Lys Phe Lys Ser Asn Cys Tyr Ser Phe
1250 1255 1260
Ser Thr Val Leu Asp Ser Met Ser Phe Glu Ala Ala His Glu Phe Cys
1265 1270 1275 1280
Lys Lys Glu Gly Ser Asn Leu Leu Thr Ile Lys Asp Glu Ala Glu Asn
1285 1290 1295
Ala Phe Leu Leu Glu Glu Leu Phe Ala Phe Gly Ser Ser Val Gln Met
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Val Trp Leu Asn Ala Gln Phe Asp Gly Asn Asn Glu Thr Ile Lys Trp
1315 1320 1325
Phe Asp Gly Thr Pro Thr Asp Gln Ser Asn Trp Gly Ile Arg Lys Pro
1330 1335 1340
Asp Thr Asp Tyr Phe Lys Pro His His Cys Val Ala Leu Arg Ile Pro
1345 1350 1355 1360
Glu Gly Leu Trp Gln Leu Ser Pro Cys Gln Glu Lys Lys Gly Phe Ile
1365 1370 1375
Cys Lys Met Glu Ala Asp Ile His Thr Ala Glu Ala Leu Pro Glu Lys
1380 1385 1390
Gly Pro Ser His Ser Ile Ile Pro Leu Ala Val Val Leu Thr Leu Ile
1395 1400 1405
Val Ile Val Ala Ile Cys Thr Leu Ser Phe Cys Ile Tyr Lys His Asn
1410 1415 1420
Gly Gly Phe Phe Arg Arg Leu Ala Gly Phe Arg Asn Pro Tyr Tyr Pro
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35 40 45
Ala Gln Gly Glu Ala Glu Ala Pro Thr Leu Tyr Leu Trp Lys Thr Gly
50 55 60
Pro Trp Gly Arg Cys Met Gly Asp Glu Cys Gly Pro Gly Gly Ile Gln
65 70 75 80
Thr Arg Ala Val Trp Cys Ala His Val Glu Gly Trp Thr Thr Leu His
85 90 95
Thr Asn Cys Lys Gln Ala Glu Arg Pro Asn Asn Gln Gln Asn Cys Phe
100 105 110
Lys Val Cys Asp Trp His Lys Glu Leu Tyr Asp Trp Arg Leu Gly Pro
115 120 125
Trp Asn Gln Cys Gln Pro Val Ile Ser Lys Ser Leu Glu Lys Pro Leu
130 135 140
Glu Cys Ile Lys Gly Glu Glu Gly Ile Gln Val Arg Glu Ile Ala Cys
145 150 155 160
Ile Gln Lys Asp Lys Asp Ile Pro Ala Glu Asp Ile Ile Cys Glu Tyr
165 170 175
Phe Glu Pro Lys Pro Leu Leu Glu Gln Ala Cys Leu Ile Pro Cys Gln
180 185 190
Gln Asp Cys Ile Val Ser Glu Phe Ser Ala Trp Ser Glu Cys Ser Lys
195 200 205
Thr Cys Gly Ser Gly Leu Gln His Arg Thr Arg His Val Val Ala Pro
210 215 220
Pro Gln Phe Gly Gly Ser Gly Cys Pro Asn Leu Thr Glu Phe Gln Val
225 230 235 240
Cys Gln Ser Ser Pro Cys Glu Ala Glu Glu Leu Arg Tyr Ser Leu His
245 250 255
Val Gly Pro Trp Ser Thr Cys Ser Met Pro His Ser Arg Gln Val Arg
260 265 270
Gln Ala Arg Arg Arg Gly Lys Asn Lys Glu Arg Glu Lys Asp Arg Ser
275 280 285

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

595 600 605

Glu Glu Val Asp Arg Gln Leu Cys Arg Asp Ala Ile Phe Pro Ile Pro

610 615 620

Val Ala Cys Asp Ala Pro Cys Pro Lys Asp Cys Val Leu Ser Thr Trp

625 630 635 640

Ser Thr Trp Ser Ser Cys Ser His Thr Cys Ser Gly Lys Thr Thr Glu

645 650 655

Gly Lys Gln Ile Arg Ala Arg Ser Ile Leu Ala Tyr Ala Gly Glu Glu

660 665 670

Gly Gly Ile Arg Cys Pro Asn Ser Ser Ala Leu Gln Glu Val Arg Ser

675 680 685

Cys Asn Glu His Pro Cys Thr Val Tyr His Trp Gln Thr Gly Pro Trp

690 695 700

Gly Gln Cys Ile Glu Asp Thr Ser Val Ser Ser Phe Asn Thr Thr Thr

705 710 715 720

Thr Trp Asn Gly Glu Ala Ser Cys Ser Val Gly Met Gln Thr Arg Lys

725 730 735

Val Ile Cys Val Arg Val Asn Val Gly Gln Val Gly Pro Lys Lys Cys

740 745 750

Pro Glu Ser Leu Arg Pro Glu Thr Val Arg Pro Cys Leu Leu Pro Cys

755 760 765

Lys Lys Asp Cys Ile Val Thr Pro Tyr Ser Asp Trp Thr Ser Cys Pro

770 775 780

Ser Ser Cys Lys Glu Gly Asp Ser Ser Ile Arg Lys Gln Ser Arg His

785 790 795 800

Arg Val Ile Ile Gln Leu Pro Ala Asn Gly Gly Arg Asp Cys Thr Asp

805 810 815

Pro Leu Tyr Glu Glu Lys Ala Cys Glu Ala Pro Gln Ala Cys Gln Ser

820 825 830

Tyr Arg Trp Lys Thr His Lys Trp Arg Arg Cys Gln Leu Val Pro Trp

835 840 845

Ser Val Gln Gln Asp Ser Pro Gly Ala Gln Glu Gly Cys Gly Pro Gly

850 855 860

Arg Gln Ala Arg Ala Ile Thr Cys Arg Lys Gln Asp Gly Gly Gln Ala

865 870 875 880

Gly Ile His Glu Cys Leu Gln Tyr Ala Gly Pro Val Pro Ala Leu Thr

885 890 895

Gln Ala Cys Gln Ile Pro Cys Gln Asp Asp Cys Gln Leu Thr Ser Trp

900 905 910

Ser Lys Phe Ser Ser Cys Asn Gly Asp Cys Gly Ala Val Arg Thr Arg
915 920 925
Lys Arg Thr Leu Val Gly Lys Ser Lys Lys Lys Glu Lys Cys Lys Asn
930 935 940
Ser His Leu Tyr Pro Leu Ile Glu Thr Gln Tyr Cys Pro Cys Asp Lys
945 950 955 960
Tyr Asn Ala Gln Pro Val Gly Asn Trp Ser Asp Cys Ile Leu Pro Glu
965 970 975
Gly Lys Val Glu Val Leu Leu Gly Met Lys Val Gln Gly Asp Ile Lys
980 985 990
Glu Cys Gly Gln Gly Tyr Arg Tyr Gln Ala Met Ala Cys Tyr Asp Gln
995 1000 1005
Asn Gly Arg Leu Val Glu Thr Ser Arg Cys Asn Ser His Gly Tyr Ile
1010 1015 1020
Glu Glu Ala Cys Ile Ile Pro Cys Pro Ser Asp Cys Lys Leu Ser Glu
1025 1030 1035 1040
Trp Ser Asn Trp Ser Arg Cys Ser Lys Ser Cys Gly Ser Gly Val Lys
1045 1050 1055
Val Arg Ser Lys Trp Leu Arg Glu Lys Pro Tyr Asn Gly Gly Arg Pro
1060 1065 1070
Cys Pro Lys Leu Asp His Val Asn Gln Ala Gln Val Tyr Glu Val Val
1075 1080 1085
Pro Cys His Ser Asp Cys Asn Gln Tyr Leu Trp Val Thr Glu Pro Trp
1090 1095 1100
Ser Ile Cys Lys Val Thr Phe Val Asn Met Arg Glu Asn Cys Gly Glu
1105 1110 1115 1120
Gly Val Gln Thr Arg Lys Val Arg Cys Met Gln Asn Thr Ala Asp Gly
1125 1130 1135
Pro Ser Glu His Val Glu Asp Tyr Leu Cys Asp Pro Glu Glu Met Pro
1140 1145 1150
Leu Gly Ser Arg Val Cys Lys Leu Pro Cys Pro Glu Asp Cys Val Ile
1155 1160 1165
Ser Glu Trp Gly Pro Trp Thr Gln Cys Val Leu Pro Cys Asn Gln Ser
1170 1175 1180
Ser Phe Arg Gln Arg Ser Ala Asp Pro Ile Arg Gln Pro Ala Asp Glu
1185 1190 1195 1200
Gly Arg Ser Cys Pro Asn Ala Val Glu Lys Glu Pro Cys Asn Leu Asn
1205 1210 1215
Lys Asn Cys Tyr His Tyr Asp Tyr Asn Val Thr Asp Trp Ser Thr Cys

1220 1225 1230

Gln Leu Ser Glu Lys Ala Val Cys Gly Asn Gly Ile Lys Thr Arg Met

1235 1240 1245

Leu Asp Cys Val Arg Ser Asp Gly Lys Ser Val Asp Leu Lys Tyr Cys

1250 1255 1260

Glu Ala Leu Gly Leu Glu Lys Asn Trp Gln Met Asn Thr Ser Cys Met

1265 1270 1275 1280

Val Glu Cys Pro Val Asn Cys Gln Leu Ser Asp Trp Ser Pro Trp Ser

1285 1290 1295

Glu Cys Ser Gln Thr Cys Gly Leu Thr Gly Lys Met Ile Arg Arg Arg

1300 1305 1310

Thr Val Thr Gln Pro Phe Gln Gly Asp Gly Arg Pro Cys Pro Ser Leu

1315 1320 1325

Met Asp Gln Ser Lys Pro Cys Pro Val Lys Pro Cys Tyr Arg Trp Gln

1330 1335 1340

Tyr Gly Gln Trp Ser Pro Cys Gln Val Gln Glu Ala Gln Cys Gly Glu

1345 1350 1355 1360

Gly Thr Arg Thr Arg Asn Ile Ser Cys Val Val Ser Asp Gly Ser Ala

1365 1370 1375

Asp Asp Phe Ser Lys Val Val Asp Glu Glu Phe Cys Ala Asp Ile Glu

1380 1385 1390

Leu Ile Ile Asp Gly Asn Lys Asn Met Val Leu Glu Glu Ser Cys Ser

1395 1400 1405

Gln Pro Cys Pro Gly Asp Cys Tyr Leu Lys Asp Trp Ser Ser Trp Ser

1410 1415 1420

Leu Cys Gln Leu Thr Cys Val Asn Gly Glu Asp Leu Gly Phe Gly Gly

1425 1430 1435 1440

Ile Gln Val Arg Ser Arg Pro Val Ile Ile Gln Glu Leu Glu Asn Gln

1445 1450 1455

His Leu Cys Pro Glu Gln Met Leu Glu Thr Lys Ser Cys Tyr Asp Gly

1460 1465 1470

Gln Cys Tyr Glu Tyr Lys Trp Met Ala Ser Ala Trp Lys Gly Ser Ser

1475 1480 1485

Arg Thr Val Trp Cys Gln Arg Ser Asp Gly Ile Asn Val Thr Gly Gly

1490 1495 1500

Cys Leu Val Met Ser Gln Pro Asp Ala Asp Arg Ser Cys Asn Pro Pro

1505 1510 1515 1520

Cys Ser Gln Pro His Ser Tyr Cys Ser Glu Thr Lys Thr Cys His Cys

1525 1530 1535

Glu Glu Gly Tyr Thr Glu Val Met Ser Ser Asn Ser Thr Leu Glu Gln
1540 1545 1550
Cys Thr Leu Ile Pro Val Val Val Leu Pro Thr Met Glu Asp Lys Arg
1555 1560 1565
Gly Asp Val Lys Thr Ser Arg Ala Val His Pro Thr Gln Pro Ser Ser
1570 1575 1580
Asn Pro Ala Gly Arg Gly Arg Thr Trp Phe Leu Gln Pro Phe Gly Pro
1585 1590 1595 1600
Asp Gly Arg Leu Lys Thr Trp Val Tyr Gly Val Ala Ala Gly Ala Phe
1605 1610 1615
Val Leu Leu Ile Phe Ile Val Ser Met Ile Tyr Leu Ala Cys Lys Lys
1620 1625 1630
Pro Lys Lys Pro Gln Arg Arg Gln Asn Asn Arg Leu Lys Pro Leu Thr
1635 1640 1645
Leu Ala Tyr Asp Gly Asp Ala Asp Met
1650 1655

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

Thr Asp Ser Ala Arg Ile Gln Arg Thr Val Phe Val Phe Tyr Tyr Phe
130 135 140
Phe Ser Pro Asn Ile Ser Ile Pro Asn Cys Gly Gly Tyr Leu Asp Thr
145 150 155 160
Leu Glu Gly Ser Phe Thr Ser Pro Asn Tyr Pro Lys Pro His Pro Glu
165 170 175
Leu Ala Tyr Cys Val Trp His Ile Gln Val Glu Lys Asp Tyr Lys Ile
180 185 190
Lys Leu Asn Phe Lys Glu Ile Phe Leu Glu Ile Asp Lys Gln Cys Lys
195 200 205
Phe Asp Phe Leu Ala Ile Tyr Asp Gly Pro Ser Thr Asn Ser Gly Leu
210 215 220
Ile Gly Gln Val Cys Gly Arg Val Thr Pro Thr Phe Glu Ser Ser Ser
225 230 235 240
Asn Ser Leu Thr Val Val Leu Ser Thr Asp Tyr Ala Asn Ser Tyr Arg
245 250 255
Gly Phe Ser Ala Ser Tyr Thr Ser Ile Tyr Ala Glu Asn Ile Asn Thr
260 265 270
Thr Ser Leu Thr Cys Ser Ser Asp Arg Met Arg Val Ile Ile Ser Lys
275 280 285
Ser Tyr Leu Glu Ala Phe Asn Ser Asn Gly Asn Asn Leu Gln Leu Lys
290 295 300
Asp Pro Thr Cys Arg Pro Lys Leu Ser Asn Val Val Glu Phe Ser Val
305 310 315 320
Pro Leu Asn Gly Cys Gly Thr Ile Arg Lys Val Glu Asp Gln Ser Ile
325 330 335
Thr Tyr Thr Asn Ile Ile Thr Phe Ser Ala Ser Ser Thr Ser Glu Val
340 345 350
Ile Thr Arg Gln Lys Gln Leu Gln Ile Ile Val Lys Cys Glu Met Gly
355 360 365
His Asn Ser Thr Val Glu Ile Ile Tyr Ile Thr Glu Asp Asp Val Ile
370 375 380
Gln Ser Gln Asn Ala Leu Gly Lys Tyr Asn Thr Ser Met Ala Leu Phe
385 390 395 400
Glu Ser Asn Ser Phe Glu Lys Thr Ile Leu Glu Ser Pro Tyr Tyr Val
405 410 415
Asp Leu Asn Gln Thr Leu Phe Val Gln Val Ser Leu His Thr Ser Asp
420 425 430
Pro Asn Leu Val Val Phe Leu Asp Thr Cys Arg Ala Ser Pro Thr Ser

435 440 445

Asp Phe Ala Ser Pro Thr Tyr Asp Leu Ile Lys Ser Gly Cys Ser Arg
450 455 460

Asp Glu Thr Cys Lys Val Tyr Pro Leu Phe Gly His Tyr Gly Arg Phe
465 470 475 480

Gln Phe Asn Ala Phe Lys Phe Leu Arg Ser Met Ser Ser Val Tyr Leu
485 490 495

Gln Cys Lys Val Leu Ile Cys Asp Ser Ser Asp His Gln Ser Arg Cys
500 505 510

Asn Gln Gly Cys Val Ser Arg Ser Lys Arg Asp Ile Ser Ser Tyr Lys
515 520 525

Trp Lys Thr Asp Ser Ile Ile Gly Pro Ile Arg Leu Lys Arg Asp Arg
530 535 540

Ser Ala Ser Gly Asn Ser Gly Phe Gln His Glu Thr His Ala Glu Glu
545 550 555 560

Thr Pro Asn Gln Pro Phe Asn Ser Val His Leu Phe Ser Phe Met Val
565 570 575

Leu Ala Leu Asn Val Val Thr Val Ala Thr Ile Thr Val Arg His Phe
580 585 590

Val Asn Gln Arg Ala Asp Tyr Lys Tyr Gln Lys Leu Gln Asn Tyr
595 600 605

<210> 12
<211> 530
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<400> 12

Met Glu Arg Met Val Gly Ser Gly Leu Leu Trp Leu Ala Leu Val Ser
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Cys Ile Leu Thr Gln Ala Ser Ala Val Gln Arg Gly Tyr Gly Asn Pro
20 25 30

Ile Glu Ala Ser Ser Tyr Gly Leu Asp Leu Asp Cys Gly Ala Pro Gly
35 40 45

Thr Pro Glu Ala His Val Cys Phe Asp Pro Cys Gln Asn Tyr Thr Leu
50 55 60

Leu Asp Glu Pro Phe Arg Ser Thr Glu Asn Ser Ala Gly Ser Gln Gly
65 70 75 80

Cys Asp Lys Asn Met Ser Gly Trp Tyr Arg Phe Val Gly Glu Gly Gly

85 90 95

Val Arg Met Ser Glu Thr Cys Val Gln Val His Arg Cys Gln Thr Asp

100 105 110

Ala Pro Met Trp Leu Asn Gly Thr His Pro Ala Leu Gly Asp Gly Ile

115 120 125

Thr Asn His Thr Ala Cys Ala His Trp Ser Gly Asn Cys Cys Phe Trp

130 135 140

Lys Thr Glu Val Leu Val Lys Ala Cys Pro Gly Gly Tyr His Val Tyr

145 150 155 160

Arg Leu Glu Gly Thr Pro Trp Cys Asn Leu Arg Tyr Cys Thr Asp Pro

165 170 175

Ser Thr Val Glu Asp Lys Cys Glu Lys Ala Cys Arg Pro Glu Glu Glu

180 185 190

Cys Leu Ala Leu Asn Ser Thr Trp Gly Cys Phe Cys Arg Gln Asp Leu

195 200 205

Asn Ser Ser Asp Val His Ser Leu Gln Pro Gln Leu Asp Cys Gly Pro

210 215 220

Arg Glu Ile Lys Val Lys Val Asp Lys Cys Leu Leu Gly Gly Leu Gly

225 230 235 240

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

245 250 255

Ile Leu Gln Thr Glu Glu Arg Asn Trp Val Ser Val Thr Ser Pro Val

260 265 270

Gln Ala Ser Ala Cys Arg Asn Ile Leu Glu Arg Asn Gln Thr His Ala

275 280 285

Ile Tyr Lys Asn Thr Leu Ser Leu Val Asn Asp Phe Ile Ile Arg Asp

290 295 300

Thr Ile Leu Asn Ile Asn Phe Gln Cys Ala Tyr Pro Leu Asp Met Lys

305 310 315 320

Val Ser Leu Gln Ala Ala Leu Gln Pro Ile Val Ser Ser Leu Asn Val

325 330 335

Ser Val Asp Gly Asn Gly Glu Phe Ile Val Arg Met Ala Leu Phe Gln

340 345 350

Asp Gln Asn Tyr Thr Asn Pro Tyr Glu Gly Asp Ala Val Glu Leu Ser

355 360 365

Val Glu Ser Val Leu Tyr Val Gly Ala Ile Leu Glu Gln Gly Asp Thr

370 375 380

Ser Arg Phe Asn Leu Val Leu Arg Asn Cys Tyr Ala Thr Pro Thr Glu

385 390 395 400

Asp Lys Ala Asp Leu Val Lys Tyr Phe Ile Ile Arg Asn Ser Cys Ser
405 410 415
Asn Gln Arg Asp Ser Thr Ile His Val Glu Glu Asn Gly Gln Ser Ser
420 425 430
Glu Ser Arg Phe Ser Val Gln Met Phe Met Phe Ala Gly His Tyr Asp
435 440 445
Leu Val Phe Leu His Cys Glu Ile His Leu Cys Asp Ser Leu Asn Glu
450 455 460
Gln Cys Gln Pro Ser Cys Ser Arg Ser Gln Val Arg Ser Glu Val Pro
465 470 475 480
Ala Ile Asp Leu Ala Arg Val Leu Asp Leu Gly Pro Ile Thr Arg Arg
485 490 495
Gly Ala Gln Ser Pro Gly Val Met Asn Gly Thr Pro Ser Thr Ala Gly
500 505 510
Phe Leu Val Ala Trp Pro Met Val Leu Leu Thr Val Leu Leu Ala Trp
515 520 525
Leu Phe
530

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<211> 368
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Met Glu Pro Gly Gln Pro Arg Glu Pro Gln Glu Pro Arg Glu Pro Gly
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Pro Gly Ala Glu Thr Ala Ala Ala Pro Val Trp Glu Glu Ala Lys Ile
20 25 30
Phe Tyr Asp Asn Leu Ala Pro Lys Lys Lys Pro Lys Ser Pro Lys Pro
35 40 45
Gln Asn Ala Val Thr Ile Ala Val Ser Ser Arg Ala Leu Phe Arg Met
50 55 60
Asp Glu Glu Gln Gln Ile Tyr Thr Glu Gln Gly Val Glu Glu Tyr Val
65 70 75 80
Arg Tyr Gln Leu Glu His Glu Asn Glu Pro Phe Ser Pro Gly Pro Ala
85 90 95
Phe Pro Phe Val Lys Ala Leu Glu Ala Val Asn Arg Arg Leu Arg Glu
100 105 110

Leu Tyr Pro Asp Ser Glu Asp Val Phe Asp Ile Val Leu Met Thr Asn
115 120 125
Asn His Ala Gln Val Gly Val Arg Leu Ile Asn Ser Ile Asn His Tyr
130 135 140
Asp Leu Phe Ile Glu Arg Phe Cys Met Thr Gly Gly Asn Ser Pro Ile
145 150 155 160
Cys Tyr Leu Lys Ala Tyr His Thr Asn Leu Tyr Leu Ser Ala Asp Ala
165 170 175
Glu Lys Val Arg Glu Ala Ile Asp Glu Gly Ile Ala Ala Ala Thr Ile
180 185 190
Phe Ser Pro Ser Arg Asp Val Val Val Ser Gln Ser Gln Leu Arg Val
195 200 205
Ala Phe Asp Gly Asp Ala Val Leu Phe Ser Asp Glu Ser Glu Arg Ile
210 215 220
Val Lys Ala His Gly Leu Asp Arg Phe Phe Glu His Glu Lys Ala His
225 230 235 240
Glu Asn Lys Pro Leu Ala Gln Gly Pro Leu Lys Gly Phe Leu Glu Ala
245 250 255
Leu Gly Arg Leu Gln Lys Lys Phe Tyr Ser Lys Gly Leu Arg Leu Glu
260 265 270
Cys Pro Ile Arg Thr Tyr Leu Val Thr Ala Arg Ser Ala Ala Ser Ser
275 280 285
Gly Ala Arg Ala Leu Lys Thr Leu Arg Ser Trp Gly Leu Glu Thr Asp
290 295 300
Glu Ala Leu Phe Leu Ala Gly Ala Pro Lys Gly Pro Leu Leu Glu Lys
305 310 315 320
Ile Arg Pro His Ile Phe Phe Asp Asp Gln Met Phe His Val Ala Gly
325 330 335
Ala Gln Glu Met Gly Thr Val Ala Ala His Val Pro Tyr Gly Val Ala
340 345 350
Gln Thr Pro Arg Arg Thr Ala Pro Ala Lys Gln Ala Pro Ser Ala Gln
355 360 365

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<400> 14

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

305 310 315 320
Ala Lys Glu Leu Asp Val Lys Ile Lys Asn Val Ile Arg Asn Val His
325 330 335
Ile Leu Leu Lys Gln Ile Ser Gly Thr Asp Gly Glu Gly Asn Asn Val
340 345 350
Pro Ser Gly Asp Phe Ser Arg Glu Trp Ala Glu Ala Gln Arg Met Met
355 360 365
Arg Glu Leu Arg Asn Arg Asn Phe Gly Lys His Leu Arg Glu Ala Glu
370 375 380
Ala Asp Lys Arg Glu Ser Gln Leu Leu Leu Asn Arg Ile Arg Thr Trp
385 390 395 400
Gln Lys Thr His Gln Gly Glu Asn Asn Gly Leu Ala Asn Ser Ile Arg
405 410 415
Asp Ser Leu Asn Glu Tyr Glu Ala Lys Leu Ser Asp Leu Arg Ala Arg
420 425 430
Leu Gln Glu Ala Ala Ala Gln Ala Lys Gln Ala Asn Gly Leu Asn Gln
435 440 445
Glu Asn Glu Arg Ala Leu Gly Ala Ile Gln Arg Gln Val Lys Glu Ile
450 455 460
Asn Ser Leu Gln Ser Asp Phe Thr Lys Tyr Leu Thr Thr Ala Asp Ser
465 470 475 480
Ser Leu Leu Gln Thr Asn Ile Ala Leu Gln Leu Met Glu Lys Ser Gln
485 490 495
Lys Glu Tyr Glu Lys Leu Ala Ala Ser Leu Asn Glu Ala Arg Gln Glu
500 505 510
Leu Ser Asp Lys Val Arg Glu Leu Ser Arg Ser Ala Gly Lys Thr Ser
515 520 525
Leu Val Glu Glu Ala Glu Lys His Ala Arg Ser Leu Gln Glu Leu Ala
530 535 540
Lys Gln Leu Glu Glu Ile Lys Arg Asn Ala Ser Gly Asp Glu Leu Val
545 550 555 560
Arg Cys Ala Val Asp Ala Ala Thr Ala Tyr Glu Asn Ile Leu Asn Ala
565 570 575
Ile Lys Ala Ala Glu Asp Ala Ala Asn Arg Ala Ala Ser Ala Ser Glu
580 585 590
Ser Ala Leu Gln Thr Val Ile Lys Glu Asp Leu Pro Arg Lys Ala Lys
595 600 605
Thr Leu Ser Ser Asn Ser Asp Lys Leu Leu Asn Glu Ala Lys Met Thr
610 615 620

Gln Lys Lys Leu Lys Gln Glu Val Ser Pro Ala Leu Asn Asn Leu Gln
625 630 635 640
Gln Thr Leu Asn Ile Val Thr Val Gln Lys Glu Val Ile Asp Thr Asn
645 650 655
Leu Thr Thr Leu Arg Asp Gly Leu His Gly Ile Gln Arg Gly Asp Ile
660 665 670
Asp Ala Met Ile Ser Ser Ala Lys Ser Met Val Arg Lys Ala Asn Asp
675 680 685
Ile Thr Asp Glu Val Leu Asp Gly Leu Asn Pro Ile Gln Thr Asp Val
690 695 700
Glu Arg Ile Lys Asp Thr Tyr Gly Arg Thr Gln Asn Glu Asp Phe Lys
705 710 715 720
Lys Ala Leu Thr Asp Ala Asp Asn Ser Val Asn Lys Leu Thr Asn Lys
725 730 735
Leu Pro Asp Leu Trp Arg Lys Ile Glu Ser Ile Asn Gln Gln Leu Leu
740 745 750
Pro Leu Gly Asn Ile Ser Asp Asn Met Asp Arg Ile Arg Glu Leu Ile
755 760 765
Gln Gln Ala Arg Asp Ala Ala Ser Lys Val Ala Val Pro Met Arg Phe
770 775 780
Asn Gly Lys Ser Gly Val Glu Val Arg Leu Pro Asn Asp Leu Glu Asp
785 790 795 800
Leu Lys Gly Tyr Thr Ser Leu Ser Leu Phe Leu Gln Arg Pro Asn Ser
805 810 815
Arg Glu Asn Gly Gly Thr Glu Asn Met Phe Val Met Tyr Leu Gly Asn
820 825 830
Lys Asp Ala Ser Arg Asp Tyr Ile Gly Met Ala Val Val Asp Gly Gln
835 840 845
Leu Thr Cys Val Tyr Asn Leu Gly Asp Arg Glu Ala Glu Leu Gln Val
850 855 860
Asp Gln Ile Leu Thr Lys Ser Glu Thr Lys Glu Ala Val Met Asp Arg
865 870 875 880
Val Lys Phe Gln Arg Ile Tyr Gln Phe Ala Arg Leu Asn Tyr Thr Lys
885 890 895
Gly Ala Thr Ser Ser Lys Pro Glu Thr Pro Gly Val Tyr Asp Met Asp
900 905 910
Gly Arg Asn Ser Asn Thr Leu Leu Asn Leu Asp Pro Glu Asn Val Val
915 920 925
Phe Tyr Val Gly Gly Tyr Pro Pro Asp Phe Lys Leu Pro Ser Arg Leu

930 935 940

Ser Phe Pro Pro Tyr Lys Gly Cys Ile Glu Leu Asp Asp Leu Asn Glu
945 950 955 960

Asn Val Leu Ser Leu Tyr Asn Phe Lys Lys Thr Phe Asn Leu Asn Thr
965 970 975

Thr Glu Val Glu Pro Cys Arg Arg Arg Lys Glu Glu Ser Asp Lys Asn
980 985 990

Tyr Phe Glu Gly Thr Gly Tyr Ala Arg Val Pro Thr Gln Pro His Ala
995 1000 1005

Pro Ile Pro Thr Phe Gly Gln Thr Ile Gln Thr Thr Val Asp Arg Gly
1010 1015 1020

Leu Leu Phe Phe Ala Glu Asn Gly Asp Arg Phe Ile Ser Leu Asn Ile
1025 1030 1035 1040

Glu Asp Gly Lys Leu Met Val Arg Tyr Lys Leu Asn Ser Glu Leu Pro
1045 1050 1055

Lys Glu Arg Gly Val Gly Asp Ala Ile Asn Asn Gly Arg Asp His Ser
1060 1065 1070

Ile Gln Ile Lys Ile Gly Lys Leu Gln Lys Arg Met Trp Ile Asn Val
1075 1080 1085

Asp Val Gln Asn Thr Ile Ile Asp Gly Glu Val Phe Asp Phe Ser Thr
1090 1095 1100

Tyr Tyr Leu Gly Gly Ile Pro Ile Ala Ile Arg Glu Arg Phe Asn Ile
1105 1110 1115 1120

Ser Thr Pro Ala Phe Arg Gly Cys Met Lys Asn Leu Lys Lys Thr Ser
1125 1130 1135

Gly Val Val Arg Leu Asn Asp Thr Val Gly Val Thr Lys Lys Cys Ser
1140 1145 1150

Glu Asp Trp Lys Leu Val Arg Ser Ala Ser Phe Ser Arg Gly Gly Gln
1155 1160 1165

Leu Ser Phe Thr Asp Leu Gly Leu Pro Pro Thr Asp His Leu Gln Ala
1170 1175 1180

Ser Phe Gly Phe Gln Thr Phe Gln Pro Ser Gly Ile Leu Leu Asp His
1185 1190 1195 1200

Gln Thr Trp Thr Arg Asn Leu Gln Val Thr Leu Glu Asp Gly Tyr Ile
1205 1210 1215

Glu Leu Ser Thr Ser Asp Ser Gly Ser Pro Ile Phe Lys Ser Pro Gln
1220 1225 1230

Thr Tyr Met Asp Gly Leu Leu His Tyr Val Ser Val Ile Ser Asp Asn
1235 1240 1245

Ser Gly Leu Arg Leu Leu Ile Asp Asp Gln Leu Leu Arg Asn Ser Lys
1250 1255 1260

Arg Leu Lys His Ile Ser Ser Ser Arg Gln Ser Leu Arg Leu Gly Gly
1265 1270 1275 1280

Ser Asn Phe Glu Gly Cys Ile Ser Asn Val Phe Val Gln Arg Leu Ser
1285 1290 1295

Leu Ser Pro Glu Val Leu Asp Leu Thr Ser Asn Ser Leu Lys Arg Asp
1300 1305 1310

Val Ser Leu Gly Gly Cys Ser Leu Asn Lys Pro Pro Phe Leu Met Leu
1315 1320 1325

Leu Lys Gly Ser Thr Arg Phe Asn Lys Thr Lys Thr Phe Arg Ile Asn
1330 1335 1340

Gln Leu Leu Gln Asp Thr Pro Val Ala Ser Pro Arg Ser Val Lys Val
1345 1350 1355 1360

Trp Gln Asp Ala Cys Ser Pro Leu Pro Lys Thr Gln Ala Asn His Gly
1365 1370 1375

Ala Leu Gln Phe Gly Asp Ile Pro Thr Ser His Leu Leu Phe Lys Leu
1380 1385 1390

Pro Gln Glu Leu Leu Lys Pro Arg Ser Gln Phe Ala Val Asp Met Gln
1395 1400 1405

Thr Thr Ser Ser Arg Gly Leu Val Phe His Thr Gly Thr Lys Asn Ser
1410 1415 1420

Phe Met Ala Leu Tyr Leu Ser Lys Gly Arg Leu Val Phe Ala Leu Gly
1425 1430 1435 1440

Thr Asp Gly Lys Lys Leu Arg Ile Lys Ser Lys Glu Lys Cys Asn Asp
1445 1450 1455

Gly Lys Trp His Thr Val Val Phe Gly His Asp Gly Glu Lys Gly Arg
1460 1465 1470

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Glu Glu Lys Trp Gln His Arg Val Val Glu Gln lle Pro Lys Glu Val
50 55 60
Gln Phe Gln Pro Pro Gly Ala Pro Leu Glu Lys Glu Lys Ser Gln Gln
65 70 75 80
Cys Tyr Ser Glu Tyr Phe Ser Gln Thr Ser Thr Glu Leu Gln lle Thr
85 90 95
Phe Asp Glu Thr Asn Pro lle Thr Arg Leu Ser Glu lle Glu Lys lle
100 105 110
Arg Asp Gln Ala Leu Asn Asn Ser Arg Pro Pro Val Arg Tyr Gln Asp
115 120 125
Asn Ala Cys Glu Met Glu Leu Val Lys Val Leu Thr Pro Leu Glu lle
130 135 140
Ala Lys Asn Lys Gln Tyr Asp Met His Thr Glu Val Thr Thr Leu Lys
145 150 155 160
Gln Glu Lys Asn Pro Val Pro Ser Ala Glu Glu Trp Met Leu Glu Gly
165 170 175

Cys Arg Ala Ser Gly Gly Leu Lys Lys Gly Asp Phe Leu Lys Lys Gly
180 185 190
Leu Glu Pro Glu Thr Phe Gln Asn Phe Asp Gly Asp His Ala Cys Ser
195 200 205
Val Arg Asp Asp Glu Phe Lys Phe Gln Gly Leu Arg His Thr Val Thr
210 215 220
Ala Arg Gln Leu Val Glu Ala Lys Leu Leu Asp Met Arg Thr Ile Glu
225 230 235 240
Gln Leu Arg Leu Gly Leu Lys Thr Val Glu Glu Val Gln Lys Thr Leu
245 250 255
Asn Lys Phe Leu Thr Lys Ala Thr Ser Ile Ala Gly Leu Tyr Leu Glu
260 265 270
Ser Thr Lys Glu Lys Ile Ser Phe Ala Ser Ala Ala Glu Arg Ile Ile
275 280 285
Ile Asp Lys Met Val Ala Leu Ala Phe Leu Glu Ala Gln Ala Ala Thr
290 295 300
Gly Phe Ile Ile Asp Pro Ile Ser Gly Gln Thr Tyr Ser Val Glu Asp
305 310 315 320
Ala Val Leu Lys Gly Val Val Asp Pro Glu Phe Arg Ile Arg Leu Leu
325 330 335
Glu Ala Glu Lys Ala Ala Val Gly Tyr Ser Tyr Ser Ser Lys Thr Leu
340 345 350
Ser Val Phe Gln Ala Met Glu Asn Arg Met Leu Asp Arg Gln Lys Gly
355 360 365
Lys His Ile Leu Glu Ala Gln Ile Ala Ser Gly Gly Val Ile Asp Pro
370 375 380
Val Arg Gly Ile Arg Val Pro Pro Glu Ile Ala Leu Gln Gln Gly Leu
385 390 395 400
Leu Asn Asn Ala Ile Leu Gln Phe Leu His Glu Pro Ser Ser Asn Thr
405 410 415
Arg Val Phe Pro Asn Pro Asn Asn Lys Gln Ala Leu Tyr Tyr Ser Glu
420 425 430
Leu Leu Arg Met Cys Val Phe Asp Val Glu Ser Gln Cys Phe Leu Phe
435 440 445
Pro Phe Gly Glu Arg Asn Ile Ser Asn Leu Asn Val Lys Lys Thr His
450 455 460
Arg Ile Ser Val Val Asp Thr Lys Thr Gly Ser Glu Leu Thr Val Tyr
465 470 475 480
Glu Ala Phe Gln Arg Asn Leu Ile Glu Lys Ser Ile Tyr Leu Glu Leu

485 490 495
Ser Gly Gln Gln Tyr Gln Trp Lys Glu Ala Met Phe Phe Glu Ser Tyr
500 505 510
Gly His Ser Ser His Met Leu Thr Asp Thr Lys Thr Gly Leu His Phe
515 520 525
Asn Ile Asn Glu Ala Ile Glu Gln Gly Thr Ile Asp Lys Ala Leu Val
530 535 540
Lys Lys Tyr Gln Glu Gly Leu Ile Thr Leu Thr Glu Leu Ala Asp Ser
545 550 555 560
Leu Leu Ser Arg Leu Val Pro Lys Lys Asp Leu His Ser Pro Val Ala
565 570 575
Gly Tyr Trp Leu Thr Ala Ser Gly Glu Arg Ile Ser Val Leu Lys Ala
580 585 590
Ser Arg Arg Asn Leu Val Asp Arg Ile Thr Ala Leu Arg Cys Leu Glu
595 600 605
Ala Gln Val Ser Thr Gly Gly Ile Ile Asp Pro Leu Thr Gly Lys Lys
610 615 620
Tyr Arg Val Ala Glu Ala Leu His Arg Gly Leu Val Asp Glu Gly Phe
625 630 635 640
Ala Gln Gln Leu Arg Gln Cys Glu Leu Val Ile Thr Gly Ile Gly His
645 650 655
Pro Ile Thr Asn Lys Met Met Ser Val Val Glu Ala Val Asn Ala Asn
660 665 670
Ile Ile Asn Lys Glu Met Gly Ile Arg Cys Leu Glu Phe Gln Tyr Leu
675 680 685
Thr Gly Gly Leu Ile Glu Pro Gln Val His Ser Arg Leu Ser Ile Glu
690 695 700
Glu Ala Leu Gln Val Gly Ile Ile Asp Val Leu Ile Ala Thr Lys Leu
705 710 715 720
Lys Asp Gln Lys Ser Tyr Val Arg Asn Ile Ile Cys Pro Gln Thr Lys
725 730 735
Arg Lys Leu Thr Tyr Lys Glu Ala Leu Glu Lys Ala Asp Phe Asp Phe
740 745 750
His Thr Gly Leu Lys Leu Leu Glu Val Ser Glu Pro Leu Met Thr Gly
755 760 765
Ile Ser Ser Leu Tyr Tyr Ser Ser Leu Leu Glu His His His His His
770 775 780
His His His
785

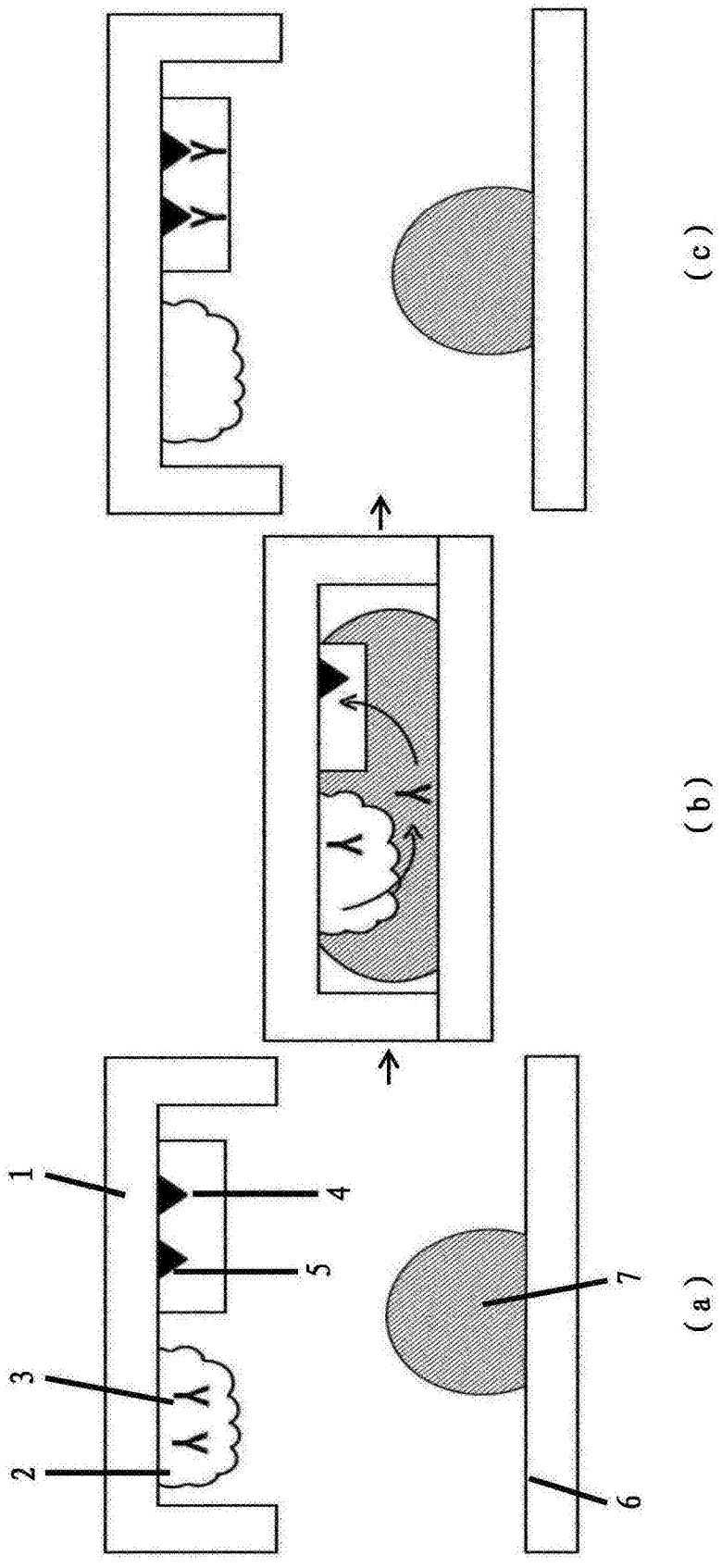

图1

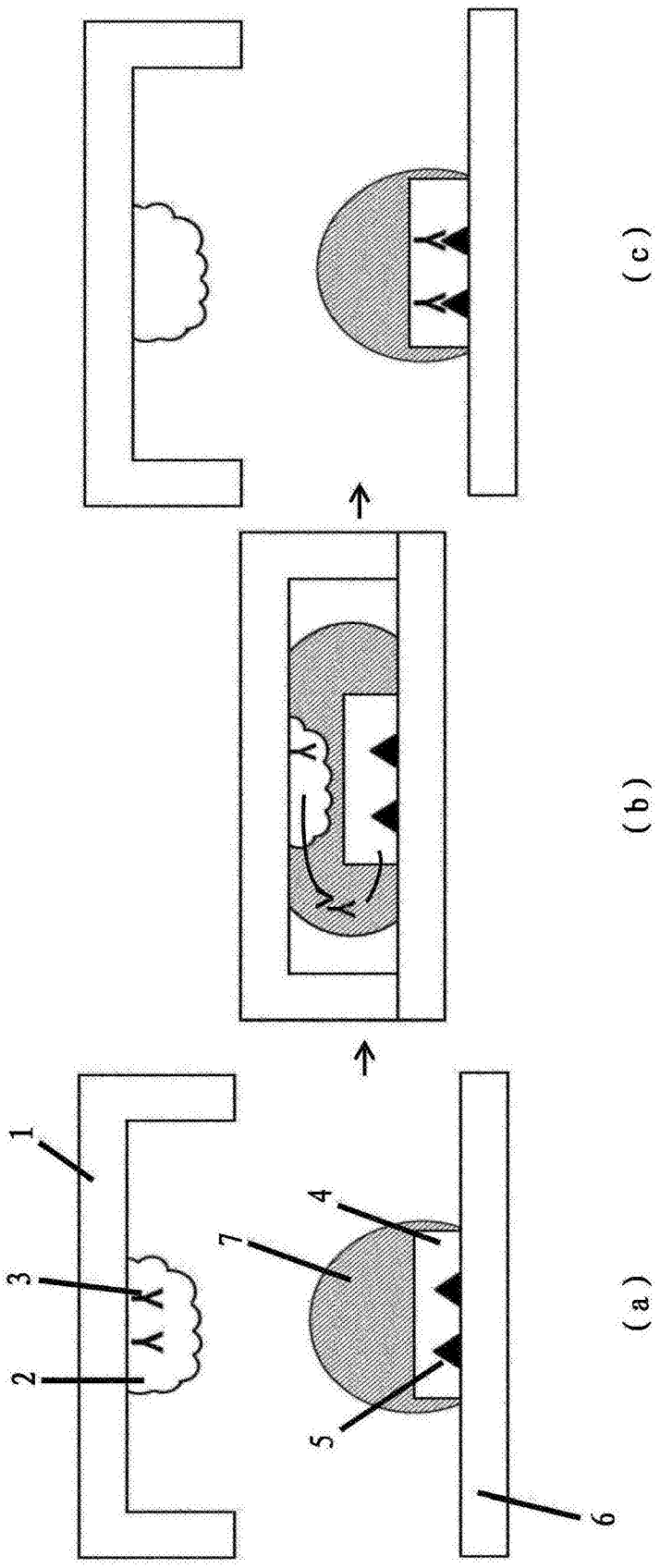

图2

图3

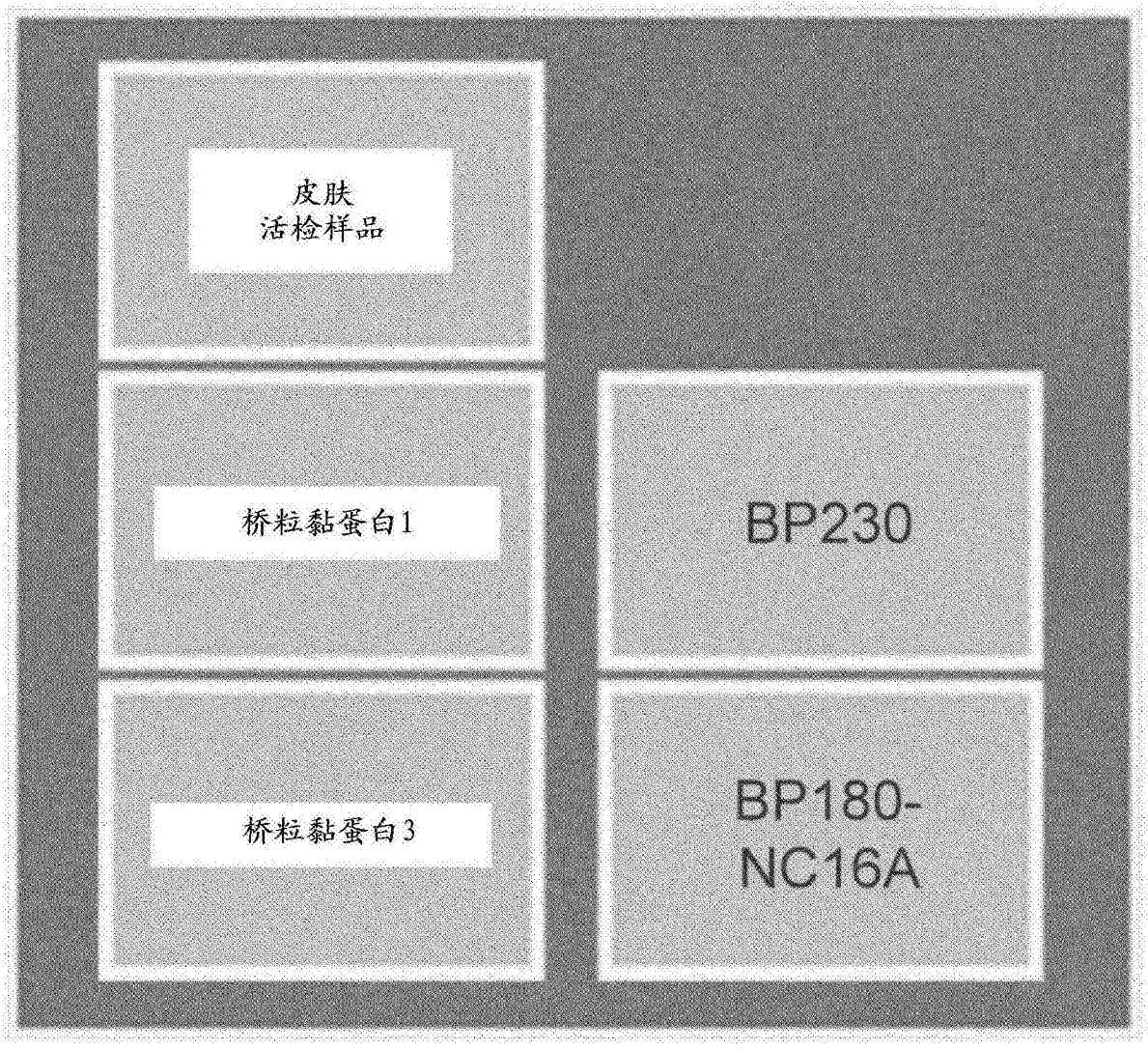

图4

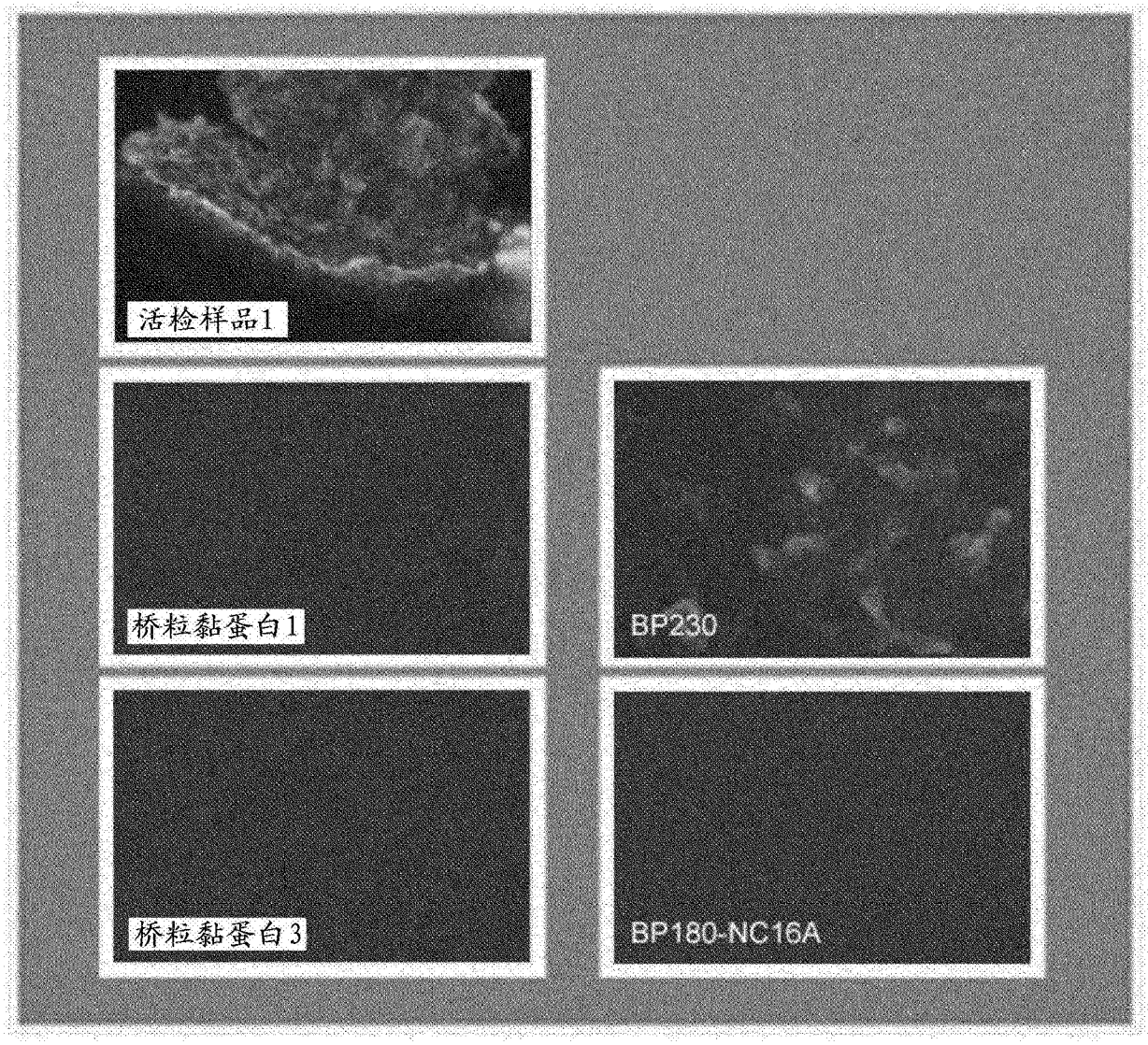

图5

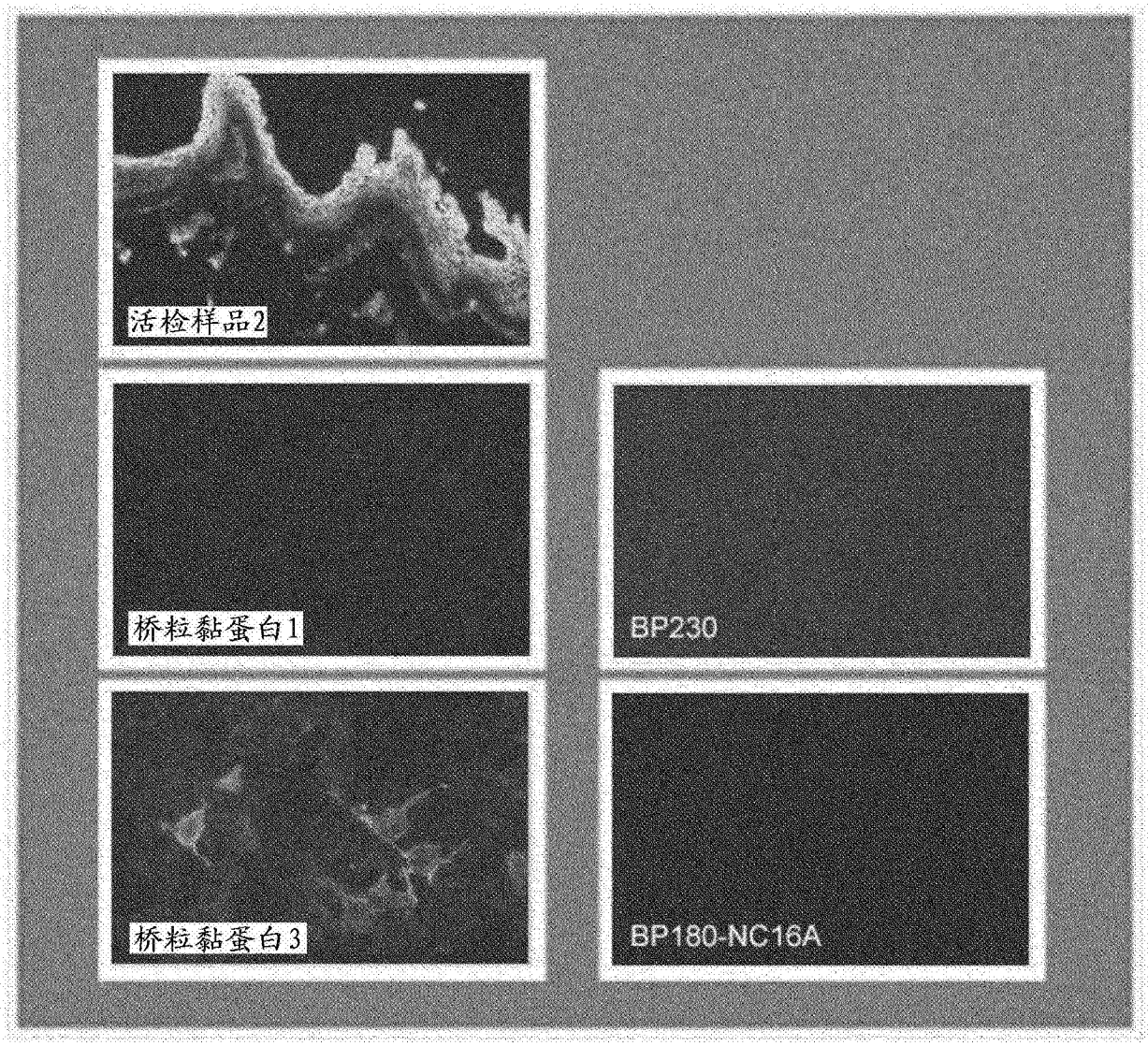

图6

专利名称(译)	诊断共孵育测定法		
公开(公告)号	CN107525919A	公开(公告)日	2017-12-29
申请号	CN201710475732.X	申请日	2017-06-21
[标]申请(专利权)人(译)	欧蒙医学诊断技术有限公司		
申请(专利权)人(译)	欧蒙医学诊断技术有限公司		
当前申请(专利权)人(译)	欧蒙医学诊断技术有限公司		
[标]发明人	W斯托克尔 B 特格 A 杨克		
发明人	W·斯托克尔 B·特格 A·杨克		
IPC分类号	G01N33/533 G01N33/536 G01N33/577		
CPC分类号	G01N33/533 G01N33/536 G01N33/577 B01L3/00 B01L3/50 B01L2300/046 B01L2300/047 B01L2300/0636 B01L2300/0819 B01L2300/0822 G01N33/564 G01N2800/06 B01L3/5085 B01L2400/0406 G01N1/2813 G01N33/5091 G01N33/5302 G01N33/54393 G01N33/549 G01N33/558 G01N33/567 G01N33/56966		
代理人(译)	张小勇		
优先权	2016001395 2016-06-21 EP		
外部链接	Espacenet SIPO		

摘要(译)

本发明涉及一种诊断疾病的方法，所述方法包括使供体组织切片与能够从所述供体组织切片提取抗体的液体接触，并使所述液体与包含抗原的受体材料接触，然后检测包括所述抗体和所述抗原的复合物，以及包含供体组织切片和包含抗原的受体材料的诊断上有用的载体。