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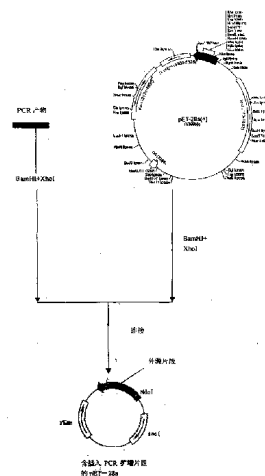
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[54] 发明名称 非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒

[57] 摘要

本发明公开了一种非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒。它提供了非典型性肺炎病毒特异蛋白质，或其具有结合人免疫球蛋白之免疫活性的片段或衍生物；并利用该通式蛋白质免疫检测人非典型性肺炎病毒的方法及试剂盒。本发明解决了临床对非典型性肺炎病毒感染者的早期快速诊断问题，具有较高的准确性，为临床检测本病提供了快速、准确的诊断方法和试剂盒。



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1、一种非典型性肺炎病毒特异蛋白质，其特征在于：它包括非典型性肺炎病毒的蛋白质和经修饰的重组非典型性肺炎病毒蛋白质，或其具有结合人免疫球蛋白之免疫活性的片段或衍生物，具有通式Seq. ID No. 1— Seq. ID No. 118的氨基酸序列的蛋白质。

2、根据权利要求1所述的蛋白质相对应的抗体，其特征在于：所述说的蛋白质相对应的抗是单克隆抗体，或是多克隆抗体。

3、一种非典型性肺炎病毒特异蛋白质临床检测方法，其特征在于其具体步骤如下：

1) 制备如权利要求1所述的蛋白质；

通过本领域技术人员周知的PCR扩增技术和重组DNA技术，具体如下：

A、设计相应的PCR引物，同时在使用PCR引物中添加编码相应数目氨基酸残基对应的碱基，分别从临床非典型性肺炎病毒标准株中扩增编码上述蛋白质的DNA片段；

B、将所得PCR产物导入合适的表达载体，构建重组DNA；

C、用所得重组DNA转化合适的宿主细胞，在适于目标蛋白质表达的条件下培养如此转化的宿主细胞；

D、回收并纯化目标蛋白质；

2) 用纯化的如权利要求1所述的蛋白质，或如权利要求2所述的抗体包被一种固相支持物；所说的固相支持物包括本领域周知的有机和无机多聚物，为葡聚糖、天然或经修饰的纤维素、聚乙烯、聚苯乙烯；或用胶体金的方法包被到试纸上；

3) 用比色法、分光光度法或荧光法测定的酶标记如权利要求1所述的蛋白质或权利要求2所述的抗体，或权利要求2所述的抗体的第二抗体(IgG、IgM、IgA)；标记酶包括氧化还原酶类，为催化酶、过氧化物酶、辣根过氧化物酶、葡萄糖氧化酶、 β -半乳糖苷酶；碱性磷酸酶；乙酰胆碱酯酶；使用胶体金，或放射性同位素作为标记；

4) 将待检测个体的生物学样本及由步骤3) 标记的蛋白质加入经包被的固相支持物上，在适合形成抗原抗体免疫结合复合物的条件下孵育一段时间；

5) 适度清洗去除任何未结合的非典型性肺炎病毒抗体；

6) 定量检测孵育混合物中抗原抗体免疫复合物的存在。

4、一种非典型性肺炎病毒特异蛋白质临床检测的试剂盒，其特征在于包括：

1) 包被有纯化的如权利要求1所述的蛋白质的固相支持物；相应的经标记的如权利要求1所述的蛋白质和使用该试剂盒的说明；

2) 包被有纯化的如权利要求2所述的抗体的固相支持物；相应的经标记的如权利要求2所述的抗体和使用该试剂盒的说明；

3) 包被有纯化的如权利要求1所述的蛋白质的固相支持物；如上文所述标记的第二抗体，该第二抗体可以是抗IgG抗体和/或抗IgM抗体和使用该试剂盒的说明；

5、根据权利要求4所述的一种非典型性肺炎病毒特异蛋白质临床检测的试剂盒，其特征在于：所说的快速检测非典型性肺炎病毒的试纸，是由喷涂过如权利要求1所述的蛋白或权利要求2所述的抗体，及喷涂有胶体金的试纸基质粘合而成；

在上述检测试剂盒中，经标记的非典型性肺炎病毒蛋白质是液体的形式包装在容器中，或便于重构的冻干物的形式置于的包装中。在后一种情况中，检测试剂盒中还优选含有用于重构经标记的非典型性肺炎病毒蛋白质的缓冲液；

在上述检测试剂盒中，还含有提供判断检测结果的相应的范围。

非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒

技术领域

本发明涉及人类医学卫生领域，具体地说是一种非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒。

背景技术

非典型性肺炎病毒，即 SARS 病毒是一种引起严重急性呼吸道疾病综合症 (Severe Acute Respiratory Syndrome, SARS)，又称非典型性肺炎的病毒，属冠状病毒 (coronavirus) 类病毒，基因组共含 29, 737 个核苷酸。造成感染严重时可致人于死，病死率高达 4.9%。主要通过近距离空气飞沫和密切接触传播。该病毒目前还没有有效的免疫化学检测方法，只能根据临床症状（高热、干咳、肺部 X 光检测有阴影等）来诊断，受到很大的局限，只能在受感染者发病后才能作出诊断，且不易与其它肺炎和一般流感区别。本发明根据该病毒的基因组序列分析结果，选取该病毒特有蛋白质和多肽片断，建立一种检测非典型性肺炎病毒抗体的免疫学方法和含有它们的试剂盒。

发明内容

本发明的目的是提供一种非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒。

本发明采用的技术方案如下：

1、一种非典型性肺炎病毒特异蛋白质，它包括非典型性肺炎病毒的蛋白质和经修饰的重组非典型性肺炎病毒蛋白质，或其具有结合人免疫球蛋白之免疫活性的片段或衍生物，具有通式Seq. ID No. 1-- Seq. ID No. 118的氨基酸序列的蛋白质。

所说的蛋白质相对应的抗体；是单克隆抗体，或是多克隆抗体。

2、一种非典型性肺炎病毒特异蛋白质临床检测方法，其具体步骤如下：

1) 制备上述的蛋白质；

通过本领域技术人员周知的PCR扩增技术和重组DNA技术，流程见附图1，具体如下：

A、设计相应的PCR引物，同时在所用PCR引物中添加编码相应数目氨基酸残基对应的碱基，分别从临床非典型性肺炎病毒标准株中扩增编码上述蛋白质

的DNA片段；

B、将所得PCR产物导入合适的表达载体，构建重组DNA；

C、用所得重组DNA转化合适的宿主细胞，在适于目标蛋白质表达的条件下培养如此转化的宿主细胞；

D、回收并纯化目标蛋白质；

2) 用纯化上述的蛋白质或抗体包被一种固相支持物；所说的固相支持物包括本领域周知的有机和无机多聚物，为葡聚糖、天然或经修饰的纤维素、聚乙烯、聚苯乙烯；或用胶体金的方法包被到试纸上；

3) 用比色法、分光光度法或荧光法测定的酶标记的蛋白质或抗体，或抗体的第二抗体(IgG、IgM、IgA)；标记酶包括但不局限于氧化还原酶类，为催化酶、过氧化物酶、辣根过氧化物酶、葡萄糖氧化酶、 β -半乳糖苷酶；碱性磷酸酶；乙酰胆碱酯酶；使用胶体金，或放射性同位素作为标记；

4) 将待检测个体的生物学样本及由步骤3)标记的蛋白质加入经包被的固相支持物上，在适合形成抗原抗体免疫结合复合物的条件下孵育一段时间；所述的“适合形成抗原抗体免疫复合物的条件”是指在适当的温度及时间条件下孵育抗原与含抗体的样品的混合物，例如在约0-50摄氏度，标记约30分钟至约48小时。

5) 适度清洗去除任何未结合的非典型性肺炎病毒抗体；所用清洗液一般为pH约5-8的磷酸盐缓冲溶液。

6) 定量检测孵育混合物中抗原抗体免疫复合物的存在。可采用多种本领域周知的方法定量检测抗原抗体复合物的形成，如以过氧化物溶液为A液及联苯胺类物质为B液的底物与带有含标记的辣根过氧化物酶的抗原抗体免疫复合物作用，显色后在波长为450nm(参比波长630nm)酶标仪读取吸光值；将所得读数与阴性对照的读数加以比较，判断所测样本的结果。

3、一种非典型性肺炎病毒特异蛋白质临床检测的试剂盒，包括：

1) 包被有纯化的蛋白质的固相支持物；相应的经标记的蛋白质和使用该试剂盒的说明(双抗原夹心法)；

2) 包被有纯化的抗体的固相支持物；相应的经标记的抗体和使用该试剂盒的说明(双抗体夹心法)；

3) 包被有纯化的蛋白质的固相支持物；相应的第二抗体，该第二抗体是抗

IgG抗体和/或抗IgM抗体和使用该试剂盒的说明（Elisa法）。

所说的快速检测非典型性肺炎病毒的试纸，是由喷涂过的蛋白或抗体，及喷涂有胶体金的试纸基质粘合而成；

在上述检测试剂盒中，经标记的非典型性肺炎病毒蛋白质是液体的形式包装在容器中，或便于重构的冻干物的形式置于的包装中。在后一种情况中，检测试剂盒中还优选含有用于重构经标记的非典型性肺炎病毒蛋白质的缓冲液；

在上述检测试剂盒中，还含有提供判断检测结果的相应的范围。

本发明与背景技术相比具有的有益效果是：它解决了临床对非典型性肺炎病毒感染者的早期快速诊断问题，具有较高的准确性，为临床检测本病提供了快速、准确的诊断方法和试剂盒。

附图说明

图1是将PCR产物与载体连接构建重组DNA示意图；

图2是高压液相色谱（HPLC）图。

具体实施方式

实施例1、

一、制备蛋白质：

A、设计相应的PCR引物，同时在所用PCR引物中添加编码相应数目氨基酸残基对应的碱基，其中之一的引物如下：A1L：CGGGATCCGAACCTTTAAAATCTGTGTAGC A1R：CCGCTCGAGATTTCTGCAACCAGCTCAAC

分别从临床非典型性肺炎病毒标准株中提取RNA和扩增编码上述蛋白质的DNA片段；提取RNA的方法可采用普通分子生物学实验手册，如“分子克隆”介绍的方法，也可以采用商业试剂盒，如Trizol^R，按说明书进行，然后加入反向引物和逆转录酶，合成cDNA；采用商品化试剂盒，如InvitrogenTM公司的SuperScript试剂盒，按说明书步骤合成cDNA；再利用PCR技术扩增编码上述蛋白质的DNA片段。PCR反应体系的组成为一般的标准组成，包括：PCR反应缓冲液（Tris.Cl 125mM PH 8.1；(NH₄)₂SO₄ 125mM；MgCl₂ 12.5mM；BSA 1mg/ml；Formamide 25%）；反应条件指：模板变性温度为92℃-98℃，优选的：95℃，时间为10秒-10分钟，优选的：30秒；模板退火温度为45℃--65℃，优选的：52℃，时间为10秒-10分钟，优选的：30秒；模板延伸温度为65℃--85℃，优选的：70℃，时间为10秒-10分钟，优选的：40秒；循环20--40次，优选的：30次。

B、将所得PCR产物导入合适的表达载体，如附图1所示的pET-28质粒，构建

重组DNA;

1 μ g 的质粒DNA于37 $^{\circ}$ C下经NdeI和XhoI酶完全酶切, 酚/氯仿抽提, -20 $^{\circ}$ C下无水乙醇沉淀过夜。离心后沉淀溶于13 μ l H₂O。经过NdeI和XhoI酶充分酶切的PCR产物与上述预酶切的质粒连接。

C、用所得重组DNA转化合适的宿主细胞, 在适于目标蛋白质表达的条件下培养如此转化的宿主细胞;

转化至大肠杆菌 ER2566, 涂布含有 200 μ g/ml 氨苄青霉素的 LB 选择平板。37 $^{\circ}$ C过夜培养。构建过程参见图 1。所述连接及转化的方法和条件参见, J. 萨姆布雷奇等著的《分子克隆实验指南》(Molecular Cloning:A Laboratory Manual) P672-849(科学出版社, 1998)

从转化平板上挑取抗性菌落, 提取质粒并电泳检查, 筛选重组子。通过限制性内切酶 NdeI 和 XhoI 酶切分析, 筛选含有正确插入的目的基因的重组子。对所得含有插入片段的重组质粒测序, 确证在 PCR 过程中目的基因片段未发生变异或出现差错。

D、回收并纯化目标蛋白质;

将选定的重组子菌株接种于含200 μ g/ml氨苄青霉素的LB培养基中, 置于恒温摇床培养, 37 $^{\circ}$ C培养至菌液OD值约0.6后加入1mM IPTG诱导, 30 $^{\circ}$ C继续培养2小时。然后将发酵培养物离心收集菌体沉淀。用超声波将所得细菌菌体破碎后再次离心, 收集上清液。利用金属镍-Sepharose(Pharmacia公司)的亲和层析柱进行蛋白的分离纯化(洗脱条件0.02M pH 7.4 磷酸盐缓冲溶液(PBS), 洗脱速度1ml/分钟)。经充分洗涤去除非特异性结合的蛋白质后, 分离并收集与金属镍-Sepharose结合的表达蛋白, 进行SDS-PAGE电泳和高压液相色谱分析。

附图2为经纯化的蛋白质的高压液相色谱图。

二、间接法检测非典型性肺炎病毒抗体

1) 每次试验设空白对照、阴性对照和阳性对照各 1 孔。空白孔不加液体; 阴、阳性对照无需稀释直接取 50 μ l 加入阴、阳性对照孔中; 其余检测孔加入样品稀释液, 每孔 100 μ l, 再加入待测样品 10 μ l。充分混匀, 贴上封口胶, 置 37 $^{\circ}$ C温育 30 分钟。

2) 将 50 倍浓缩洗板液用双蒸水 50 倍稀释后用于洗涤板孔。

3) 弃去各孔中样品, 每孔加满洗液, 静置数秒后弃去, 重复 5 次, 拍干。

4) 每孔加入酶标抗人 IgG 工作液 100 μ l, 贴上封口胶。置 37 $^{\circ}$ C温育 20 分钟。

5) 弃去各孔中液体, 用洗板液反复洗涤 5 次, 拍干。

6) 每孔加入底物液 A 50 μ l, 再加入底物液 B 50 μ l, 轻拍混匀, 置 37 $^{\circ}$ C 避光温育 10 分钟。

7) 显色完毕后, 每孔加入终止液 50 μ l, 轻拍混匀, 立即以空白孔调零, 置酶标仪 450nm 波长下测定 OD 值 (参考波长为 630nm)。

实施例 2、双抗原夹心法检测非典型性肺炎病毒抗体

在 96 孔微孔反应板上加入预先用 0.02M pH9.6 的碳酸盐缓冲液 (CB) 稀释抗原至最适浓度的包被液, 4 $^{\circ}$ C 静置 24 小时, 加入含 0.1%TW-20 的 0.02M pH7.4 PBS 洗涤液 (PBS-T) 加满各孔, 静置数秒后弃去, 重复清洗两次。每孔加入 200 μ l 入 1% 牛血清白蛋白 (BSA) 和 1% 脱脂奶粉的 0.02M pH7.4 磷酸盐缓冲液 (PBS), 4 $^{\circ}$ C 下封闭 2 小时。弃去封闭液, 室温下晾干, 用铝薄袋封板, 4 $^{\circ}$ C 保藏待用。在微孔反应板孔中加入预先用酶标抗原稀释液 (20% 山羊血清, 1% 酪蛋白, 0.02M PBS, pH7.4) 以优化好的浓度稀释的 SARS 冠状病毒抗原相应的酶标抗原, 每孔 50 μ l, 再加入 15 个来自不同待检测的受试者血清, 37 $^{\circ}$ C 温育 60 分钟。然后用含 0.1%TW-20 的 0.02M PBS-T 洗涤液加满各孔, 静置数秒后弃去, 重复清洗 5 次。每孔中加入 50 μ l 由 0.06% 过氧化脲 (Sigma) 0.01M pH4.5 的醋酸盐缓冲液组成的酶作用底物 A 液以及 50 μ l 由 0.06%TMB (Sigma) 0.01M pH 4.5 的柠檬酸盐缓冲液组成的酶作用底物 B 液, 37 $^{\circ}$ C 温育 15 分钟。每孔中加入 50 μ l 的 2M H₂SO₄ 终止反应。在酶标仪 (Dynatech MR 4100) 上读取 450nm 处的吸光值 (以 630 为参比波长)。其中阳性对照和阴性对照均为经过临床确诊及其他可能的方法复核的人血清, 根据实预先确定的阈值判定样本结果, 高于阈值的样本为非典型性肺炎病毒阳性。

氨基酸序列表:

<110>杭州华大基因研发中心

<120>一种检测非典型性肺炎病毒抗体的试剂盒

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<170>PatentIn Version 3.1

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<213>SARS coronavirus

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10

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

Tyr Arg Gln Tyr Leu Asp Ala Tyr Asn Met Met Ile Ser Ala Gly Phe
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 35 40 45
 Thr Ile Gly Val Cys Thr Met Thr Asp Ile Ala Lys Lys Pro Thr Glu
 50 55 60
 Ser Ala Cys Ser Ser Leu Thr Val Leu Phe Asp Gly Arg Val Glu Gly 80
 65 70 75
 Gln Val Asp Leu Phe Arg Asn Ala Arg Asn Gly Val Leu Ile Thr Glu 95
 85 90
 Gly Ser Val Lys Gly Leu Thr Pro Ser Lys Gly Pro Ala Gln Ala Ser 110
 100 105 110
 Val Asn Gly Val Thr Leu Ile Gly Glu Ser Val Lys Thr Gln Phe Asn
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 Arg Met Leu Gly Arg Phe Ser Asn Phe Ser Leu Gln Val Phe Leu Thr
 35 40 45
 Ser Ser Arg Val Lys Tyr Arg Leu Leu Gln Ile Thr Ser Arg Ile Val
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 Asn Ala Ser Leu Met Leu Leu Thr Arg His Ser Lys Cys Ala Leu Ile
 65 70 75 80
 Lys Ser Leu Ser Leu Ala Gln Ser Cys Asp His Ser Thr Val Lys Ser
 85 90 95
 Ser Ser Leu Lys Ala Arg Asp Phe Thr Val Ser Val Tyr Val Ala Arg
 100 105 110
 Ser Ser Cys Asn Tyr Ser Cys Leu Leu Arg His Gln Lys Lys Pro Phe
 115 120 125
 Leu Lys Val Ile His Met Thr Gln Tyr Leu Pro Leu Arg Arg Leu Phe
 144
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 1 5 10 15
 Asp Leu Glu Asp Phe Lys Pro Arg Ser Gln Met Glu Thr Asp Phe Leu
 20 25 30
 Glu Leu Ala Met Asp Glu Phe Ile Gln Arg Tyr Lys Leu Glu Gly Tyr
 35 40 45
 Ala Phe Glu His Ile Val Tyr Gly Asp Phe Ser His Gly Gln Leu Gly
 50 55 60
 Gly Leu His Leu Met Ile Gly Leu Ala Lys Arg Ser Gln Asp Ser Pro
 65 70 75 80
 Leu Lys Leu Glu Asp Phe Ile Pro Met Asp Ser Thr Val Lys Asn Tyr
 85 90 95
 Phe Ile Thr Asp Ala Gln Thr Gly Ser Ser Lys Cys Val Cys Ser Val
 100 105 110
 Ile Asp Leu Leu Leu Asp Asp Phe Val Glu Ile Ile Lys Ser Gln Asp
 115 120 125
 Leu Ser Val Ile Ser Lys Val Val Lys Val Thr Ile Asp Tyr Ala Glu
 130 135 140
 Ile Ser Phe Met Leu Trp
 145 150
 <210>31
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<212>PRT

<213>SARS coronavirus

<400>31

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

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Cys Ala

145 146

<210>32

<211>151

<212>PRT

<213>SARS coronavirus

<400>32

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

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 Ser Leu Phe Asp Met Ser Lys
 145 150 151
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 20 25 30
 Asp Met Ile Tyr Ser Leu Leu Glu Lys Gly Arg Leu Ile Ile Arg Glu
 35 40 45
 Asn Asn Arg Val Val Val Ser Ser Asp Ile Leu Val Asn Asn Thr Asn
 50 55 60
 Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu 80
 65 70 75
 Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln 95
 85 90
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 100 105 110
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 115 120 125
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 130 135 140
 Ile Pro Phe Lys Asp
 145 149
 <210>34
 <211>152
 <212>PRT
 <213>SARS coronavirus
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 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 20 25 30
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 35 40 45
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 50 55 60
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met 80
 65 70 75

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 85 90 95
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 100 105 110
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 115 120 125
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 130 135 140
 Leu Pro Ser Gly Phe Asn Thr Leu
 145 150 152
 <210>35
 <211>152
 <212>PRT
 <213>SARS coronavirus
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 Tyr Gln Pro Ile Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr
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 Leu Lys Pro Ile Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe
 20 25 30
 Arg Ala Ile Leu Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr
 35 40 45
 Ser Ala Ala Ala Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met
 50 55 60
 Leu Lys Tyr Asp Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser
 65 70 75 80
 Gln Asn Pro Leu Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile
 85 90 95
 Asp Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val Val Pro Ser Gly
 100 105 110
 Asp Val Val Arg Phe Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu
 115 120 125
 Val Phe Asn Ala Thr Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys
 130 135 140
 Lys Ile Ser Asn Cys Val Ala Asp
 145 150 152
 <210>36
 <211>153
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 <213>SARS coronavirus
 <400>36
 Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys
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 Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe
 20 25 30

Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser
 35 40 45
 Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln
 50 55 60
 Ile Ala Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu
 65 70 75 80
 Pro Asp Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile
 85 90 95
 Asp Ala Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg
 100 105 110
 His Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe
 115 120 125
 Ser Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp
 130 135 140
 Pro Leu Asn Asp Tyr Gly Phe Tyr Thr
 145 150 153
 <210>37
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 <212>PRT
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 <400>37
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 Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val Val Val Leu Ser Phe
 20 25 30
 Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly Pro Lys Leu Ser Thr
 35 40 45
 Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn Phe Asn Gly Leu Thr
 50 55 60
 Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg Phe Gln Pro Phe Gln
 65 70 75 80
 Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp Ser Val Arg Asp Pro
 85 90 95
 Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys Ala Phe Gly Gly Val
 100 105 110
 Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser Glu Val Ala Val Leu
 115 120 125
 Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr Ala Ile His Ala Asp
 130 135 140
 Gln Leu
 145 146
 <210>38
 <211>147
 <212>PRT
 <213>SARS coronavirus

<400>38
 Cys Thr Asp Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr Pro Ala
 1 5 10 15
 Trp Arg Ile Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln Ala Gly
 20 25 30
 Cys Leu Ile Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys Asp Ile
 35 40 45
 Pro Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser Leu Leu
 50 55 60
 Arg Ser Thr Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly
 65 70 75 80
 Ala Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr
 85 90 95
 Asn Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala
 100 105 110
 Lys Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu
 115 120 125
 Cys Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn
 130 135 140
 Arg Ala Leu
 145 147
 <210>39
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 <212>PRT
 <213>SARS coronavirus
 <400>39
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 1 5 10 15
 Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val
 20 25 30
 Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn
 35 40 45
 Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe
 50 55 60
 Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe
 65 70 75 80
 Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu
 85 90 95
 Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu
 100 105 110
 Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr
 115 120 125
 Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro
 130 135 140

Phe Ala Met
 145 147
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 <213>SARS coronavirus
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 1 5 10
 Gly Glu Leu Glu Ala Leu Glu Thr Pro Val Asp Ser Phe Thr Asn Gly
 20 25 30
 Ala Ile Val Gly Thr Pro Val Cys Val Asn Gly Leu Met Leu Leu Glu
 35 40 45
 Ile Lys Asp Lys Glu Gln Tyr Cys Ala Leu Ser Pro Gly Leu Leu Ala
 50 55 60
 Thr Asn Asn Val Phe Arg Leu Lys Gly Gly Ala Pro Ile Lys Gly Val 80
 65 70 75
 Thr Phe Gly Glu Asp Thr Val Trp Glu Val Gln Gly Tyr Lys Asn Val 95
 85 90
 Arg Ile Thr Phe Glu Leu Asp Glu Arg Val Asp Lys Val Leu Asn Glu
 100 105 110
 Lys Cys Ser Val Tyr Thr Val Glu Ser Gly Thr Glu Val Thr Glu Phe
 115 120 125
 Ala Cys Val Val Ala Glu Ala Val Val Lys Thr Leu Gln Pro Val Ser
 130 135 140
 Asp Leu
 145 146
 <210>41
 <211>149
 <212>PRT
 <213>SARS coronavirus
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 Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe Ala Met Gln 15
 1 5 10
 Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn Val Leu Tyr 30
 20 25 30
 Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln
 35 40 45
 Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln
 50 55 60
 Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln 80
 65 70 75
 Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu 95
 85 90

Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp Arg Leu Ile
 100 105 110
 Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile
 115 120 125
 Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala Thr Lys Met
 130 135 140
 Ser Glu Cys Val Leu
 145 149
 <210>42
 <211>150
 <212>PRT
 <213>SARS coronavirus
 <400>42
 Arg Ala Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu
 1 5 10 15
 Gly Gln Ser Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met
 20 25 30
 Ser Phe Pro Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr
 35 40 45
 Tyr Val Pro Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys
 50 55 60
 His Glu Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn
 65 70 75 80
 Gly Thr Ser Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile
 85 90 95
 Ile Thr Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile
 100 105 110
 Gly Ile Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp
 115 120 125
 Ser Phe Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro
 130 135 140
 Asp Val Asp Leu Gly Asp
 145 150
 <210>43
 <211>120
 <212>PRT
 <213>SARS coronavirus
 <400>43
 Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile
 1 5 10 15
 Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg
 20 25 30
 Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln
 35 40 45

Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp
 50 55 60
 Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu
 65 70 75 80
 Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser
 85 90 95
 Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu
 100 105 110
 Lys Gly Val Lys Leu His Tyr Thr
 115 120
 <210>44
 <211>151
 <212>PRT
 <213>SARS coronavirus
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 Thr Leu Gly Ser Ile Thr Ala Gln Pro Val Lys Ile Asp Asn Ala Ser
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 Pro Ala Ser Thr Val His Ala Thr Ala Thr Ile Pro Leu Gln Ala Ser
 20 25 30
 Leu Pro Phe Gly Trp Leu Val Ile Gly Val Ala Phe Leu Ala Val Phe
 35 40 45
 Gln Ser Ala Thr Lys Ile Ile Ala Leu Asn Lys Arg Trp Gln Leu Ala
 50 55 60
 Leu Tyr Lys Gly Phe Gln Phe Ile Cys Asn Leu Leu Leu Phe Val
 65 70 75 80
 Thr Ile Tyr Ser His Leu Leu Leu Val Ala Ala Gly Met Glu Ala Gln
 85 90 95
 Phe Leu Tyr Leu Tyr Ala Leu Ile Tyr Phe Leu Gln Cys Ile Asn Ala
 100 105 110
 Cys Arg Ile Ile Met Arg Cys Trp Leu Cys Trp Lys Cys Lys Ser Lys
 115 120 125
 Asn Pro Leu Leu Tyr Asp Ala Asn Tyr Phe Val Cys Trp His Thr His
 130 135 140
 Asn Tyr Asp Tyr Cys Ile Pro
 145 150 151
 <210>45
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 <212>PRT
 <213>SARS coronavirus
 <400>45
 Asp Ala Asn Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr Cys
 1 5 10 15
 Ile Pro Tyr Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly Asp
 20 25 30

Gly Ile Ser Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly Tyr
 35 40 45
 Ser Glu Asp Arg His Ser Gly Val Lys Asp Tyr Val Val Val His Gly
 50 55 60
 Tyr Phe Thr Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr Thr
 65 70 75 80
 Asp Thr Gly Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu Val
 85 90 95
 Lys Asp Pro Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser Gly
 100 105 110
 Val Ala Asn Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr Thr
 115 120 125
 Thr Ser Val Pro Leu
 130 133
 <210>46
 <211>83
 <212>PRT
 <213>SARS coronavirus
 <400>46
 Gln Glu Ser Glu Tyr Glu Leu Met Tyr Ser Phe Val Ser Glu Glu Thr
 1 5 10 15
 Gly Thr Leu Ile Val Asn Ser Val Leu Leu Phe Leu Ala Phe Val Val
 20 25 30
 Phe Leu Leu Val Thr Leu Ala Ile Leu Thr Ala Leu Arg Leu Cys Ala
 35 40 45
 Tyr Cys Cys Asn Ile Val Asn Val Ser Leu Val Lys Pro Thr Val Tyr
 50 55 60
 Val Tyr Ser Arg Val Lys Asn Leu Asn Ser Ser Glu Gly Val Pro Asp
 65 70 75 80
 Leu Leu Val
 83
 <210>47
 <211>150
 <212>PRT
 <213>SARS coronavirus
 <400>47
 Leu Leu Gln Phe Ala Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile
 1 5 10 15
 Lys Leu Val Phe Leu Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe
 20 25 30
 Val Leu Ala Ala Val Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala
 35 40 45
 Ile Ala Met Ala Cys Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val
 50 55 60

Ala Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn
 65 70 75 80
 Pro Glu Thr Asn Ile Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val
 85 90 95
 Thr Arg Pro Leu Met Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile
 100 105 110
 Arg Gly His Leu Arg Met Ala Gly His Ser Leu Gly Arg Cys Asp Ile
 115 120 125
 Lys Asp Leu Pro Lys Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser
 130 135 140
 Tyr Tyr Lys Leu Gly Ala
 145 150
 <210>48
 <211>55
 <212>PRT
 <213>SARS coronavirus
 <400>48
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 1 5 10 15
 Ser Gln Arg Val Gly Thr Asp Ser Gly Phe Ala Ala Tyr Asn Arg Tyr
 20 25 30
 Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp His Ala Gly Ser Asn Asp
 35 40 45
 Asn Ile Ala Leu Leu Val Gln
 50 55
 <210>49
 <211>117
 <212>PRT
 <213>SARS coronavirus
 <400>49
 Phe Leu Thr Leu Ile Val Phe Thr Ser Cys Glu Leu Tyr His Tyr Gln
 1 5 10 15
 Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys Glu Pro Cys Pro Ser
 20 25 30
 Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro Leu Ala Asp Asn Lys
 35 40 45
 Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala Phe Ala Cys Ala Asp
 50 55 60
 Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg Ser Val Ser Pro Lys
 65 70 75 80
 Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu Leu Tyr Ser Pro Leu
 85 90 95
 Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile Leu Cys Phe Thr Ile
 100 105 110

Lys Arg Lys Thr Glu
 115 117
 <210>50
 <211>55
 <212>PRT
 <213>SARS coronavirus
 <400>50
 Ala Ile Pro Cys Phe Asn Asn Ala Tyr Tyr Ile Leu Val Phe Thr Arg
 1 5 10 15
 Asn Pro Gly Ser Arg Arg Thr Leu Tyr Gln Ser Leu Asn Glu His Glu
 20 25 30
 Thr Ser His Cys Phe Asp Leu Tyr Phe Ser Met Gln Leu His Met His
 35 40 45
 Cys Ser Thr Ala Leu Cys Ile
 50 55
 <210>51
 <211>147
 <212>PRT
 <213>SARS coronavirus
 <400>51
 Ala Val Val Lys Thr Leu Gln Pro Val Ser Asp Leu Leu Thr Asn Met
 1 5 10 15
 Gly Ile Asp Leu Asp Glu Trp Ser Val Ala Thr Phe Tyr Leu Phe Asp
 20 25 30
 Asp Ala Gly Glu Glu Asn Phe Ser Ser Arg Met Tyr Cys Ser Phe Tyr
 35 40 45
 Pro Pro Asp Glu Glu Glu Glu Asp Asp Ala Glu Cys Glu Glu Glu Glu
 50 55 60
 Ile Asp Glu Thr Cys Glu His Glu Tyr Gly Thr Glu Asp Asp Tyr Gln
 65 70 75 80
 Gly Leu Pro Leu Glu Phe Gly Ala Ser Ala Glu Thr Val Arg Val Glu
 85 90 95
 Glu Glu Glu Glu Glu Asp Trp Leu Asp Asp Thr Thr Glu Gln Ser Glu
 100 105 110
 Ile Glu Pro Glu Pro Glu Pro Thr Pro Glu Glu Pro Val Asn Gln Phe
 115 120 125
 Thr Gly Tyr Leu Lys Leu Thr Asp Asn Val Ala Ile Lys Cys Val Asp
 130 135 140
 Ile Val Lys
 145 147
 <210>52
 <211>146
 <212>PRT
 <213>SARS coronavirus
 <400>52

Asn Glu Gln Ile Lys Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg
 1 5 10 15
 Ser Ala Pro Arg Ile Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn
 20 25 30
 Asn Gln Asn Gly Gly Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro
 35 40 45
 Gln Gly Leu Pro Asn Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln
 50 55 60
 His Gly Lys Glu Glu Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile
 65 70 75 80
 Asn Thr Asn Ser Gly Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala
 85 90 95
 Thr Arg Arg Val Arg Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro
 100 105 110
 Arg Trp Tyr Phe Tyr Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro
 115 120 125
 Tyr Gly Ala Asn Lys Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala
 130 135 140
 Leu Asn
 145 146
 <210>53
 <211>147
 <212>PRT
 <213>SARS coronavirus
 <400>53
 Asn Lys Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr
 1 5 10 15
 Pro Lys Asp His Ile Gly Thr Arg Asn Pro Asn Asn Asn Ala Ala Thr
 20 25 30
 Val Leu Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala
 35 40 45
 Glu Gly Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg
 50 55 60
 Ser Arg Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn
 65 70 75 80
 Ser Pro Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu
 85 90 95
 Leu Leu Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys
 100 105 110
 Gly Gln Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu
 115 120 125
 Ala Ser Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn
 130 135 140
 Val Thr Gln
 145 147

<210>54
 <211>146
 <212>PRT
 <213>SARS coronavirus
 <400>54
 Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn Val Thr Gln Ala Phe
 1 5 10 15
 Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly Asp Gln Asp
 20 25 30
 Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln Ile Ala Gln
 35 40 45
 Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg Ile Gly Met
 50 55 60
 Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr His Gly Ala Ile Lys
 65 70 75 80
 Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn Val Ile Leu Leu Asn
 85 90 95
 Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu Pro Lys Lys
 100 105 110
 Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro Gln Arg Gln
 115 120 125
 Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp Met Asp Asp
 130 135 140
 Phe Ser
 145 146
 <210>55
 <211>51
 <212>PRT
 <213>SARS coronavirus
 <400>55
 Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro Gln Arg Gln
 1 5 10 15
 Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp Met Asp Asp
 20 25 30
 Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser Ala Asp Ser
 35 40 45
 Thr Gln Ala
 50 51
 <210>56
 <211>51
 <212>PRT
 <213>SARS coronavirus
 <400>56
 Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro Gln Arg Gln
 1 5 10 15

Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp Met Asp Asp
 20 25 30
 Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser Ala Asp Ser
 35 40 45
 Thr Gln Ala
 50 51
 <210>57
 <211>150
 <212>PRT
 <213>SARS coronavirus
 <400>57
 Tyr Leu Lys Leu Thr Asp Asn Val Ala Ile Lys Cys Val Asp Ile Val
 1 5 10 15
 Lys Glu Ala Gln Ser Ala Asn Pro Met Val Ile Val Asn Ala Ala Asn
 20 25 30
 Ile His Leu Lys His Gly Gly Gly Val Ala Gly Ala Leu Asn Lys Ala
 35 40 45
 Thr Asn Gly Ala Met Gln Lys Glu Ser Asp Asp Tyr Ile Lys Leu Asn
 50 55 60
 Gly Pro Leu Thr Val Gly Gly Ser Cys Leu Leu Ser Gly His Asn Leu
 65 70 75 80
 Ala Lys Lys Cys Leu His Val Val Gly Pro Asn Leu Asn Ala Gly Glu
 85 90 95
 Asp Ile Gln Leu Leu Lys Ala Ala Tyr Glu Asn Phe Asn Ser Gln Asp
 100 105 110
 Ile Leu Leu Ala Pro Leu Leu Ser Ala Gly Ile Phe Gly Ala Lys Pro
 115 120 125
 Leu Gln Ser Leu Gln Val Cys Val Gln Thr Val Arg Thr Gln Val Tyr
 130 135 140
 Ile Ala Val Asn Asp Lys
 145 150
 <210>58
 <211>155
 <212>PRT
 <213>SARS coronavirus
 <400>58
 Leu Gln Ser Leu Gln Val Cys Val Gln Thr Val Arg Thr Gln Val Tyr
 1 5 10 15
 Ile Ala Val Asn Asp Lys Ala Leu Tyr Glu Gln Val Val Met Asp Tyr
 20 25 30
 Leu Asp Asn Leu Lys Pro Arg Val Glu Ala Pro Lys Gln Glu Glu Pro
 35 40 45
 Pro Asn Thr Glu Asp Ser Lys Thr Glu Glu Lys Ser Val Val Gln Lys
 50 55 60

Pro Val Asp Val Lys Pro Lys Ile Lys Ala Cys Ile Asp Glu Val Thr
 65 70 75 80
 Thr Thr Leu Glu Glu Thr Lys Phe Leu Thr Asn Lys Leu Leu Leu Phe
 85 90 95
 Ala Asp Ile Asn Gly Lys Leu Tyr His Asp Ser Gln Asn Met Leu Arg
 100 105 110
 Gly Glu Asp Met Ser Phe Leu Glu Lys Asp Ala Pro Tyr Met Val Gly
 115 120 125
 Asp Val Ile Thr Ser Gly Asp Ile Thr Cys Val Val Ile Pro Ser Lys
 130 135 140
 Lys Ala Gly Gly Thr Thr Glu Met Leu Ser Arg
 145 150 155
 <210>59
 <211>149
 <212>PRT
 <213>SARS coronavirus
 <400>59
 Leu Ser Thr Asn His Gly His Lys Val Val Glu Leu Val Ala Glu Met
 1 5 10 15
 Asp Gly Ile Gln Tyr Gly Arg Ser Gly Ile Thr Leu Gly Val Leu Val
 20 25 30
 Pro His Val Gly Glu Thr Pro Ile Ala Tyr Arg Asn Val Leu Leu Arg
 35 40 45
 Lys Asn Gly Asn Lys Gly Ala Gly Gly His Ser Tyr Gly Ile Asp Leu
 50 55 60
 Lys Ser Tyr Asp Leu Gly Asp Glu Leu Gly Thr Asp Pro Ile Glu Asp
 65 70 75 80
 Tyr Glu Gln Asn Trp Asn Thr Lys His Gly Ser Gly Ala Leu Arg Glu
 85 90 95
 Leu Thr Arg Glu Leu Asn Gly Gly Ala Val Thr Arg Tyr Val Asp Asn
 100 105 110
 Asn Phe Cys Gly Pro Asp Gly Tyr Pro Leu Asp Cys Ile Lys Asp Phe
 115 120 125
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 130 135 140
 Tyr Ile Glu Ser Lys
 145 149
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 Gln Gly Cys Ala Gly Tyr Thr Leu Glu Glu Ala Lys Thr Ala Leu Lys
 35 40 45
 Lys Cys Lys Ser Ala Phe Tyr Val Leu Pro Ser Glu Ala Pro Asn Ala
 50 55 60
 Lys Glu Glu Ile Leu Gly Thr Val Ser Trp Asn Leu Arg Glu Met Leu
 65 70 75 80
 Ala His Ala Glu Glu Thr Arg Lys Leu Met Pro Ile Cys Met Asp Val
 85 90 95
 Arg Ala Ile Met Ala Thr Ile Gln Arg Lys Tyr Lys Gly Ile Lys Ile
 100 105 110
 Gln Glu Gly Ile Val Asp Tyr Gly Val Arg Phe Phe Phe Tyr Thr Ser
 115 120 125
 Lys Glu Pro Val Ala Ser Ile Ile Thr Lys Leu Asn Ser Leu Asn Glu
 130 135 140
 Pro Leu Val Thr
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 Ser Leu Lys Ala Pro Ala Val Val Ser Val Ser Ser Pro Asp Ala Val
 35 40 45
 Thr Thr Tyr Asn Gly Tyr Leu Thr Ser Ser Ser Lys Thr Ser Glu Glu
 50 55 60
 His Phe Val Glu Thr Val Ser Leu Ala Gly Ser Tyr Arg Asp Trp Ser
 65 70 75 80
 Tyr Ser Gly Gln Arg Thr Glu Leu Gly Val Glu Phe Leu Lys Arg Gly
 85 90 95
 Asp Lys Ile Val Tyr His Thr Leu Glu Ser Pro Val Glu Phe His Leu
 100 105 110
 Asp Gly Glu Val Leu Ser Leu Asp Lys Leu Lys Ser Leu Leu Ser Leu
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 Arg Glu Val Lys Thr Ile Lys Val Phe Thr Thr Val Asp Asn Thr Asn
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 Leu His Thr
 145 147
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<400>62

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 Ser Leu Lys Ala Pro Ala Val Val Ser Val Ser Ser Pro Asp Ala Val
 35 40 45
 Thr Thr Tyr Asn Gly Tyr Leu Thr Ser Ser Ser Lys Thr Ser Glu Glu
 50 55 60
 His Phe Val Glu Thr Val Ser Leu Ala Gly Ser Tyr Arg Asp Trp Ser
 65 70 75 80
 Tyr Ser Gly Gln Arg Thr Glu Leu Gly Val Glu Phe Leu Lys Arg Gly
 85 90 95
 Asp Lys Ile Val Tyr His Thr Leu Glu Ser Pro Val Glu Phe His Leu
 100 105 110
 Asp Gly Glu Val Leu Ser Leu Asp Lys Leu Lys Ser Leu Leu Ser Leu
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 130 135 140
 Leu His Thr
 145 147

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

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 130 135 140
 Thr Leu Tyr Arg Ile
 145 149
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 20 25 30
 Asp Val Phe Tyr Lys Glu Thr Ser Tyr Thr Thr Thr Ile Lys Pro Val
 35 40 45
 Ser Tyr Lys Leu Asp Gly Val Thr Tyr Thr Glu Ile Glu Pro Lys Leu
 50 55 60
 Asp Gly Tyr Tyr Lys Lys Asp Asn Ala Tyr Tyr Thr Glu Gln Pro Ile 80
 65 70 75
 Asp Leu Val Pro Thr Gln Pro Leu Pro Asn Ala Ser Phe Asp Asn Phe
 85 90 95
 Lys Leu Thr Cys Ser Asn Thr Lys Phe Ala Asp Asp Leu Asn Gln Met
 100 105 110
 Thr Gly Phe Thr Lys Pro Ala Ser Arg Glu Leu Ser Val Thr Phe Phe
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 Pro Asp Leu Asn Gly Asp Val Val Ala Ile Asp Tyr Arg His Tyr Ser
 130 135 140
 Ala Ser Phe Lys
 145 148
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 Thr Lys Thr Thr Phe Lys Pro Asn Thr Trp Cys Leu Arg Cys Leu Trp
 35 40 45
 Ser Thr Lys Pro Val Asp Thr Ser Asn Ser Phe Glu Val Leu Ala Val
 50 55 60
 Glu Asp Thr Gln Gly Met Asp Asn Leu Ala Cys Glu Ser Gln Gln Pro 80
 65 70 75

Gly Val Arg Glu Leu Tyr Leu Asn Ser Ser Asn Val Thr Thr Met Asp
 35 40 45
 Phe Cys Glu Gly Ser Phe Pro Cys Ser Ile Cys Leu Ser Gly Leu Asp
 50 55 60
 Ser Leu Asp Ser Tyr Pro Ala Leu Glu Thr Ile Gln Val Thr Ile Ser
 65 70 75 80
 Ser Tyr Lys Leu Asp Leu Thr Ile Leu Gly Leu Ala Ala Glu Trp Val
 85 90 95
 Leu Ala Tyr Met Leu Phe Thr Lys Phe Phe Tyr Leu Leu Gly Leu Ser
 100 105 110
 Ala Ile Met Gln Val Phe Phe Gly Tyr Phe Ala Ser His Phe Ile Ser
 115 120 125
 Asn Ser Trp Leu Met Trp Phe Ile Ile Ser Ile Val Gln Met Ala Pro
 130 135 140
 Val Ser Ala Met Val Arg Met
 145 150 151
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 20 25 30
 Asp Glu Ser Ala Ser Lys Ser Ala Ser Val Tyr Tyr Ser Gln Leu Met
 35 40 45
 Cys Gln Pro Ile Leu Leu Leu Asp Gln Ala Leu Val Ser Asp Val Gly
 50 55 60
 Asp Ser Thr Glu Val Ser Val Lys Met Phe Asp Ala Tyr Val Asp Thr
 65 70 75 80
 Phe Ser Ala Thr Phe Ser Val Pro Met Glu Lys Leu Lys Ala Leu Val
 85 90 95
 Ala Thr Ala His Ser Glu Leu Ala Lys Gly Val Ala Leu Asp Gly Val
 100 105 110
 Leu Ser Thr Phe Val Ser Ala Ala Arg Gln Gly Val Val Asp Thr Asp
 115 120 125
 Val Asp Thr Lys Asp Val Ile Glu Cys Leu Lys Leu Ser His His Ser
 130 135 140
 Asp Leu Glu Val Thr Gly
 145 150
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<400>69

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

Ile His

145 146

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<400>70

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

Thr Thr Cys Gly Tyr Leu
 145 150
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 20 25 30
 Val Arg Glu Leu Tyr Leu Asn Ser Ser Asn Val Thr Thr Met Asp Phe
 35 40 45
 Cys Glu Gly Ser Phe Pro Cys Ser Ile Cys Leu Ser Gly Leu Asp Ser
 50 55 60
 Leu Asp Ser Tyr Pro Ala Leu Glu Thr Ile Gln Val Thr Ile Ser Ser 80
 65 70 75
 Tyr Lys Leu Asp Leu Thr Ile Leu Gly Leu Ala Ala Glu Trp Val Leu
 85 90 95
 Ala Tyr Met Leu Phe Thr Lys Phe Phe Tyr Leu Leu Gly Leu Ser Ala
 100 105 110
 Ile Met Gln Val Phe Phe Gly Tyr Phe Ala Ser His Phe Ile Ser Asn
 115 120 125
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 130 135 140
 Ser Ala Met Val Arg Met
 145 150
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 Ala Ala Leu Val Cys Tyr Ile Val Met Pro Val His Thr Leu Ser Ile
 20 25 30
 His Asp Gly Tyr Thr Asn Glu Ile Ile Gly Tyr Lys Ala Ile Gln Asp
 35 40 45
 Gly Val Thr Arg Asp Ile Ile Ser Thr Asp Asp Cys Phe Ala Asn Lys
 50 55 60
 His Ala Gly Phe Asp Ala Trp Phe Ser Gln Arg Gly Gly Ser Tyr Lys 80
 65 70 75
 Asn Asp Lys Ser Cys Pro Val Val Ala Ala Ile Ile Thr Arg Glu Ile
 85 90 95

Gly Phe Ile Val Pro Gly Leu Pro Gly Thr Val Leu Arg Ala Ile Asn
 100 105 110
 Gly Asp Phe Leu His Phe Leu Pro Arg Val Phe Ser Ala Val Gly Asn
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 130 135 140
 Ser Ala Cys Val Leu
 145 149
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 20 25 30
 Val Pro Tyr Cys Tyr Asp Thr Asn Leu Leu Glu Gly Ser Ile Ser Tyr
 35 40 45
 Ser Glu Leu Arg Pro Asp Thr Arg Tyr Val Leu Met Asp Gly Ser Ile
 50 55 60
 Ile Gln Phe Pro Asn Thr Tyr Leu Glu Gly Ser Val Arg Val Val Thr
 65 70 75 80
 Thr Phe Asp Ala Glu Tyr Cys Arg His Gly Thr Cys Glu Arg Ser Glu
 85 90 95
 Val Gly Ile Cys Leu Ser Thr Ser Gly Arg Trp Val Leu Asn Asn Glu
 100 105 110
 His Tyr Arg Ala Leu Ser Gly Val Phe Cys Gly Val Asp Ala Met Asn
 115 120 125
 Leu Ile Ala Asn Ile Phe Thr Pro Leu Val Gln Pro Val Gly Ala Leu
 130 135 140
 Asp Val Ser Ala Ser Val Val
 145 150 151
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 20 25 30
 Met Lys Phe Arg Arg Val Phe Gly Glu Tyr Asn His Val Val Ala Ala
 35 40 45

Asn Ala Leu Leu Phe Leu Met Ser Phe Thr Ile Leu Cys Leu Val Pro
 50 55 60
 Ala Tyr Ser Phe Leu Pro Gly Val Tyr Ser Val Phe Tyr Leu Tyr Leu
 65 70 75 80
 Thr Phe Tyr Phe Thr Asn Asp Val Ser Phe Leu Ala His Leu Gln Trp
 85 90 95
 Phe Ala Met Phe Ser Pro Ile Val Pro Phe Trp Ile Thr Ala Ile Tyr
 100 105 110
 Val Phe Cys Ile Ser Leu Lys His Cys His Trp Phe Phe Asn Asn Tyr
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 Leu Arg Lys Arg Val Met Phe Asn Gly Val Thr Phe Ser Thr Phe Glu
 130 135 140
 Glu Ala Ala Leu Cys Thr Phe Leu Leu Asn
 145 150 154
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 Phe Leu Leu Asn Lys Glu Met Tyr Leu Lys Leu Arg Ser Glu Thr Leu
 20 25 30
 Leu Pro Leu Thr Gln Tyr Asn Arg Tyr Leu Ala Leu Tyr Asn Lys Tyr
 35 40 45
 Lys Tyr Phe Ser Gly Ala Leu Asp Thr Thr Ser Tyr Arg Glu Ala Ala
 50 55 60
 Cys Cys His Leu Ala Lys Ala Leu Asn Asp Phe Ser Asn Ser Gly Ala
 65 70 75 80
 Asp Val Leu Tyr Gln Pro Pro Gln Thr Ser Ile Thr Ser Ala Val Leu
 85 90 95
 Gln Ser Gly Phe Arg Lys Met Ala Phe Pro Ser Gly Lys Val Glu Gly
 100 105 110
 Cys Met Val Gln Val Thr Cys Gly Thr Thr Thr Leu Asn Gly Leu Trp
 115 120 125
 Leu Asp Asp Thr Val Tyr Cys Pro Arg His Val Ile Cys Thr Ala Glu
 130 135 140
 Asp Met Leu
 145 147
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 Ile Ile Ile Asn Asn Ser Thr Asn
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 1 5 10 15
 Lys Ser Phe Glu Ile Asp Lys Gly
 20 24
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 1 5 10 15
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 20 24
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 Tyr Ala Trp Glu Arg Lys Lys
 20 23
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 1 5 10 15
 Asp Gln Leu Thr Pro Ala Trp Arg
 20 24
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 <400>86

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 1 5 10 15

Gly Ala Glu His Val Asp Thr Ser
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<210>87

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<400>87

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Asp Asp Ser Glu Pro Val Leu Lys
 20 24

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<212>PRT

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Lys Lys Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro
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<400>90

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Ser Arg Gly Asn Ser Arg Asn Ser
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<400>91

Lys Lys Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn Ser Pro Ala
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 Thr Lys Lys
 19
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 1 5 10 15
 Gln Arg Gln Lys Lys Gln
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 1 5 10 15
 Tyr Leu Gly Thr Gly Pro Glu Ala
 20 24
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 1 5 10 15
 Thr Gln Ala Phe Gly Arg Arg Gly
 20 24
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 20 25 27
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 Ile Leu Leu Asn Val Pro Leu Arg
 20 24
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 1 5 10 15
 Ala Gly His Ser Leu Gly Arg
 20 23
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 20 25 27
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 Tyr Lys Leu Asn Thr Asp His Ala Gly
 20 25
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 1 5 10 15
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe
 20 25
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 20 25 27
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 Asp Ala Val Asp Cys Ser Gln Asn
 20 24
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 1 5 10 15
 Thr Phe Phe Ser Thr Phe Lys Cys
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 Ser Tyr His Thr Val Ser Leu Leu Arg
 20 25
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Lys Lys Met Val Thr Ile Leu Leu Cys Cys Met Thr Ser Cys Cys Ser
 1 5 10 15
 Cys Leu Lys Gly Ala Cys Ser Cys
 20 24
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 1 5 10 15
 Lys Gly Val Lys Leu His Tyr Thr
 20 24
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 1 5 10 15
 Asn Thr Ala Ser Trp Phe Thr Ala
 20 24
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 1 5 10 15
 Glu Gly Ser Arg Gly Gly Ser Gln
 20 24
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 Lys Lys Gly Gly Glu Thr Ala Leu Ala Leu leu leu Leu Asp Arg Leu
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 20 25 27
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 <400>116

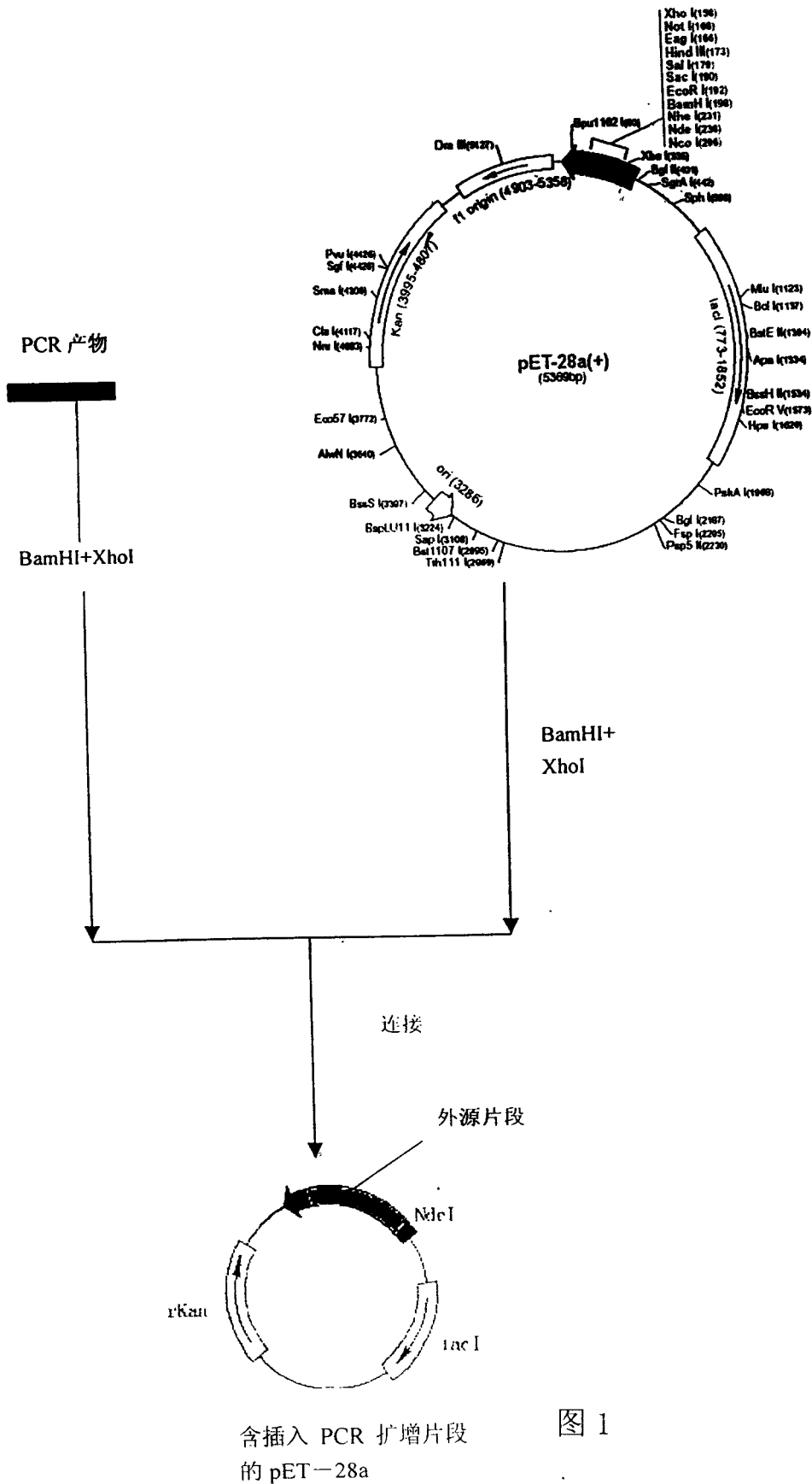


图 1

专利名称(译)	非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒		
公开(公告)号	CN1483737A	公开(公告)日	2004-03-24
申请号	CN03116657.1	申请日	2003-04-26
[标]申请(专利权)人(译)	杭州华大基因研发中心		
申请(专利权)人(译)	杭州华大基因研发中心		
当前申请(专利权)人(译)	杭州华大基因研发中心		
[标]发明人	汪建 方健秋 文洁 牟峰 陈唯军 刘斯奇 胡咏武 殷剑宁 王俊 杨焕明		
发明人	汪建 方健秋 文洁 牟峰 陈唯军 刘斯奇 胡咏武 殷剑宁 王俊 杨焕明		
IPC分类号	C07K14/165 C07K16/08 C07K19/00 C12Q1/68 G01N33/52 G01N33/531 G01N33/569 G01N33/577 G01N33/68		
其他公开文献	CN1216902C		
外部链接	Espacenet SIPO		

摘要(译)

本发明公开了一种非典型性肺炎病毒特异蛋白质和临床检测的方法及试剂盒。它提供了非典型性肺炎病毒特异蛋白质，或其具有结合人免疫球蛋白之免疫活性的片段或衍生物；并利用该通式蛋白质免疫检测人非典型性肺炎病毒的方法及试剂盒。本发明解决了临床对非典型性肺炎病毒感染者的早期快速诊断问题，具有较高的准确性，为临床检测本病提供了快速、准确的诊断方法和试剂盒。

