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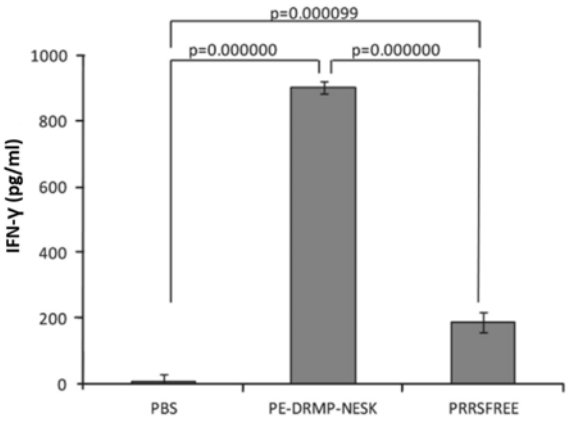
(54)发明名称

用于猪生殖与呼吸综合症及猪圆环病毒相关疾病的疫苗组合物

(57)摘要

本发明提供一种融合蛋白,其包含抗原呈递细胞(APC)结合域或CD91受体结合域、转位肽、融合抗原、内质网滞留序列以及任选的核输出信号。该融合抗原包含猪生殖与呼吸综合症病毒(PRRSV)ORF7抗原、PRRSV ORF1b抗原、PRRSV ORF6抗原以及PRRSV ORF5抗原。该融合蛋白可用于诱发抗原特异性细胞介导及体液反应。



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1.一种融合蛋白,其包含:

(a)抗原呈递细胞(APC)结合域,位于该融合蛋白的N端,其中APC结合域为绿脓杆菌外毒素A(PE)结合域;

(b)PE转位肽,长度为34-112个氨基酸残基,其包含与序列识别号:4、2、3或6相同的氨基酸序列,位于该APC结合域的C端;

(c)融合抗原,其由猪生殖与呼吸综合症病毒四种抗原的融合所组成,所述四种抗原为:

(i)猪生殖与呼吸综合症病毒(PRRSV)ORF7抗原;

(ii)PRRSV ORF1b抗原;

(iii)PRRSV ORF6抗原;及

(iv)PRRSV ORF5抗原;

(d)核输出信号,其由序列识别号:13的氨基酸序列所组成,位于所述融合抗原的C末端或位于该PE转位肽与所述融合抗原之间;

其中该融合抗原并不包含全长的ORF7、ORF6、ORF5及ORF1b的蛋白质序列;以及

(e)内质网滞留序列,当核输出信号位于该PE转位肽和所述融合抗原之间时位于所述融合抗原的C末端,或当核输出信号位于所述融合抗原的C末端时位于核输出信号的C末端。

2.如权利要求1所述的融合蛋白,其中该ORF7或ORF1b抗原位于该ORF6抗原的N端,且该ORF5抗原位于该ORF6抗原的C端。

3.如权利要求1所述的融合蛋白,其中所述融合抗原包含两个该ORF7抗原的串联重复序列。

4.如权利要求1所述的融合蛋白,其中该ORF5抗原位于该ORF6抗原的C端。

5.如权利要求1所述的融合蛋白,其中该ORF6抗原为该PRRSV ORF6的N端部分氨基酸序列,该ORF5抗原为该PRRSV ORF5的N端部分氨基酸序列,且该融合抗原并不包含该ORF6及ORF5的C端部分氨基酸序列。

6.如权利要求5所述的融合蛋白,其中该ORF1b抗原由ORF1b NSP 10的C端部分氨基酸序列及ORF1b NSP 11的N端部分氨基酸序列所组成,且该融合抗原缺乏该ORF1b的N端及C端部分氨基酸序列。

7.如权利要求1所述的融合蛋白,其中该APC结合域的氨基酸序列为序列识别号:1或32。

8.如权利要求1所述的融合蛋白,其中该PE转位肽的长度为34-61个氨基酸残基。

9.如权利要求1所述的融合蛋白,其中该内质网滞留序列包含氨基酸序列KDEL(序列识别号:15),不含氨基酸KDEL的串联重复序列。

10.如权利要求9所述的融合蛋白,其中该核输出信号及该内质网滞留序列构成融合肽,该融合肽由序列识别号:12的氨基酸序列所组成。

11.如权利要求1-10中任一项所述的融合蛋白,其氨基酸序列为序列识别号:27或28。

12.如权利要求1-10中任一项所述的融合蛋白,其中该融合抗原由下列(i)、(ii)、(iii)及(iv)的氨基酸序列所组成:

(i)序列识别号:22、23或33的氨基酸序列;

(ii)序列识别号:24的氨基酸序列;

(iii)序列识别号:25的氨基酸序列;及

(iv)序列识别号:26的氨基酸序列。

13.一种组合物,其包含:

(i)如权利要求1所述的融合蛋白;

(ii)猪圆环病毒2型(PCV2)融合蛋白,包含:

(a)抗原呈递细胞(APC)结合域或CD91受体结合域,其位于该融合蛋白的N端,其中所述APC结合域或所述CD91受体结合域为包含选自SEQ ID NO:1、8、9、10及11的氨基酸序列的多肽;

(b)PE转位肽,其长度为34-112个氨基酸残基,其包含与序列识别号:4、2、3或6相同的氨基酸序列,其位于所述APC结合域或所述CD91受体结合域的C端;及

(c)PCV2 ORF2抗原;

(d)核输出信号,其由序列识别号:13的氨基酸序列所组成,位于该PCV2 ORF2抗原的C末端或位于该PE转位肽与该PCV2 ORF2抗原之间;以及

(e)内质网滞留序列,当核输出信号位于该PE转位肽和该PCV2 ORF2抗原之间时位于PCV2融合蛋白的C末端,或当该核输出信号位于该PCV2ORF2抗原的C末端时位于该核输出信号的C末端;

其中该PCV2 ORF2抗原为PCV2 ORF 2蛋白的C端部分氨基酸序列,且该PCV2融合蛋白并不包含PCV2 ORF2蛋白的N端部分氨基酸序列。

14.如权利要求13所述的组合物,其中该猪圆环病毒2型(PCV2)融合蛋白的氨基酸序列为序列识别号:31。

15.如权利要求1-12中任一项所述的融合蛋白,在制备用于在有需要的个体中诱发针对PRRSV的抗原特异性细胞介导及体液反应的药物的用途,或者如权利要求13或14所述的组合物在制备用于在有需要的个体中诱发针对PRRSV和PCV2的抗原特异性细胞介导及体液反应的药物的用途。

用于猪生殖与呼吸综合症及猪圆环病毒相关疾病的疫苗组合物

技术领域

[0001] 本发明大致关于疫苗,且具体是关于亚单位疫苗。

背景技术

[0002] 会感染免疫细胞(例如T细胞、B细胞、树突细胞、单核细胞或巨噬细胞)的病毒包括猪生殖与呼吸综合症病毒(PRRSV)、猪圆环病毒2型(PCV2)以及人类免疫缺陷病毒(HIV)。其使得免疫细胞无法唤起免疫反应,反而载运病毒。受到上述病毒感染的动物容易受到其他病原体感染。猪生殖与呼吸综合症病毒(PRRSV)每年均对畜牧业造成重大损失。不仅猪,鸭类亦会受PRRSV感染。一般而言,受到这种病毒感染的动物并无显著症状,但其免疫力有所降低。此病毒侵袭巨噬细胞(在肺泡及脾脏中)、脑部微神经胶细胞及单核细胞,且可存在于受感染动物的血液及器官中,导致体重减轻及因二次感染造成的死亡率提高。

[0003] 美国专利第7,595,054号揭露一种作为亚单位疫苗的融合抗原,其中是将选自ORF1b的区域或ORF7的区域的单一抗原部分融合于绿脓杆菌外毒素A(Pseudomnoas exotoxin A)多肽(其缺乏细胞毒素结构域III,亦即PE(ΔIII))与内质网滞留序列之间。

[0004] 瑞宝基因股份有限公司(Reber Genetics Co.Ltd.)所命名的疫苗组合物“PRRSFREE™”包含四种不同PRRS抗原,分别称为D、M、R及P。这四种PRRS抗原分别由四种采用美国专利第7,595,054号揭露设计的不同载体表达,且能够有效诱发动物体内的细胞介导及体液免疫反应。

发明内容

[0005] 一方面,本发明提供一种猪生殖与呼吸综合症病毒(PRRSV)融合蛋白,包含:

[0006] (a)抗原呈递细胞(antigen-presenting cell,APC)结合域或CD91受体结合域,位于该融合蛋白的N端;

[0007] (b)转位肽(translocation peptide),长度为34-112个氨基酸残基,包含至少与序列识别号:4、2、3或6为90%相同的氨基酸序列,位于该APC结合域或该CD91受体结合域的C端;

[0008] (c)融合抗原,其包含:

[0009] (i)猪生殖与呼吸综合症病毒(porcine reproductive and respiratory syndrome virus,PRRSV)ORF7抗原;

[0010] (ii)PRRSV ORF1b抗原;

[0011] (iii)PRRSV ORF6抗原;及

[0012] (iv)PRRSV ORF5抗原;

[0013] (d)内质网滞留序列(endoplasmic reticulum retention sequence),位于该融合蛋白的C端;以及

[0014] (e)任选的核输出信号(nuclear export signal,NES),包含序列识别号:13的氨

基酸序列,位于所述抗原与该内质网滞留序列之间或位于该转位肽与所述抗原之间;

[0015] 其中该融合抗原并不包含全长的ORF7、ORF6、ORF5及ORF1b的蛋白质序列。

[0016] 在本发明一个实施例中,该ORF7或ORF1b抗原位于该ORF6抗原的N端,且该ORF5抗原位于该ORF6抗原的C端。

[0017] 在本发明另一个实施例中,该ORF6抗原位于该ORF5抗原的N端,且ORF6与ORF5抗原之间并无桥梁或连结。

[0018] 在本发明另一个实施例中,该融合抗原包含ORF7抗原的两个串联重复序列(tandem repeats)。

[0019] 在本发明另一个实施例中,该ORF5抗原位于该ORF6抗原的C端。

[0020] 在本发明另一个实施例中,该ORF6抗原包含PRRSV ORF6的N端部分氨基酸序列,该ORF5抗原包含PRRSV ORF5的N端部分氨基酸序列,且该融合抗原并不包含ORF6及ORF5的C端部分氨基酸序列。

[0021] 在本发明另一个实施例中,该ORF1b抗原包含ORF1b非结构蛋白(Non-structural protein,NSP)10的C端部分氨基酸序列以及ORF1b NSP 11的N端部分氨基酸序列,且该融合抗原缺乏ORF1b的N端及C端部分氨基酸序列。

[0022] 在本发明另一个实施例中,该APC结合域或该CD91受体结合域为多肽,其包含与序列识别号:1、8、9、10、11或32至少为90%相同的氨基酸序列。

[0023] 在本发明另一个实施例中,当该核输出信号(NES)存在时,该内质网滞留序列包含该氨基酸序列KDEL(序列识别号:15),且不含氨基酸KDEL的串联重复序列。

[0024] 在本发明另一个实施例中,该APC结合域或该CD91受体结合域为多肽,其包含与序列识别号:1或32至少为90%相同的氨基酸序列。

[0025] 在本发明另一个实施例中,该核输出信号及该内质网滞留序列构成融合肽,具有与序列识别号:12至少为90%相同的氨基酸序列。

[0026] 在本发明另一个实施例中,当该核输出信号(NES)不存在时,该内质网滞留序列包含序列识别号:16、17、18或19的氨基酸序列。

[0027] 在本发明另一个实施例中,该APC结合域或该CD91受体结合域为多肽,其包含与序列识别号:8至少为90%相同的氨基酸序列。

[0028] 在另一方面,本发明关于一种组合物,其包含:

[0029] (i)本发明的PRRSV融合蛋白;以及

[0030] (ii)猪圆环病毒2型(porcine circovius type 2,PCV2)融合蛋白,包含:

[0031] (a)抗原呈递细胞(APC)结合域或CD91受体结合域,位于该融合蛋白的N端;

[0032] (b)转位肽,长度为34-112个氨基酸残基,包含与序列识别号:4、2、3或6至少为90%相同的氨基酸序列,位于该APC结合域或该CD91受体结合域的C端;及

[0033] (c)PCV2ORF2抗原;

[0034] (d)内质网滞留序列,位于该融合蛋白的C端;以及

[0035] (e)核输出信号,包含序列识别号:13的氨基酸序列,位于该抗原与该内质网滞留序列之间或位于该转位肽与该抗原之间;

[0036] 其中该PCV2ORF2抗原包含PCV2ORF2蛋白的C端部分氨基酸序列,且该PCV2融合蛋白并不包含PCV2ORF2蛋白的N端部分氨基酸序列。

[0037] 在本发明另一个实施例中，该APC结合域或该CD91受体结合域不带有绿脓杆菌外毒素A(PE)结合域I的氨基酸序列。

[0038] 在本发明另一个实施例中，该转位肽的长度为34-46个氨基酸残基。

[0039] 在本发明另一个实施例中，该转位肽的长度为34-61个氨基酸残基。

[0040] 又一方面，本发明是关于一种诱发抗原特异性细胞介导及体液反应的方法，其包含对需要治疗的个体施以包含治疗上有效量的本发明融合蛋白的组合物，藉此诱发抗原特异性细胞介导及体液反应。

[0041] 再一方面，本发明是关于一种组合物的用途，该组合物包含治疗上有效量的本发明的融合蛋白或本发明的组合物，用以制造一种可在需要治疗的个体体内诱发抗原特异性细胞介导及体液反应的医药品。本发明还关于一种组合物，其包含治疗上有效量的本发明的融合蛋白或本发明的组合物，用以在需要治疗的个体体内诱发抗原特异性细胞介导及体液反应。

[0042] 或者，本发明是关于一种用以诱发抗原特异性细胞介导及体液反应的方法，包含对需要治疗的个体施以本发明的组合物，藉此诱发抗原特异性细胞介导及体液反应。

[0043] 本发明的融合抗原包含ORF7、ORF6、ORF5及ORF1b上的中和及保护性表位(neutralization and protective epitopes)，并且不含全长的ORF7、ORF6、ORF5及ORF1b的蛋白质序列。

[0044] ORF7抗原包含序列识别号:33、22或23的氨基酸序列。

[0045] ORF1b抗原包含ORF1b NSP 10的C端部分及ORF1b NSP 11的N端部分的氨基酸序列，且缺乏ORF1b的N端及C端部分。亦即，该融合抗原包含ORF1b NSP 10的C端部分氨基酸序列以及ORF1b NSP 11的N端部分氨基酸序列，但并不包含ORF1b的N端及C端部分氨基酸序列。

[0046] ORF6抗原包含PRRSV ORF6的N端部分氨基酸序列，ORF5抗原包含PRRSV ORF5的N端部分氨基酸序列，且该融合抗原并不包含ORF6及ORF5的C端部分氨基酸序列。换言之，ORF6抗原选自PRRSV ORF6的N端部分氨基酸序列，且ORF5抗原选自PRRSV ORF5的N端部分氨基酸序列。

[0047] 在本发明另一个实施例中，PRRSV ORF6的N端部分氨基酸序列为序列识别号:34，且PRRSV ORF5的N端部分氨基酸序列为序列识别号:35。

[0048] 在本发明又一个实施例中，PRRSV ORF6的N端部分氨基酸序列为序列识别号:36，且PRRSV ORF5的N端部分氨基酸序列为序列识别号:37。ORF1b抗原包含ORF1b NSP 10的C端部分氨基酸序列及ORF1b NSP 11的N端部分氨基酸序列，且该融合抗原缺乏ORF1b的N端及C端部分氨基酸序列。在本发明一个实施例中，ORF1b抗原的长度少于200个氨基酸残基，且包含序列识别号:25的氨基酸序列。在本发明另一个实施例中，该ORF1b抗原包含从PRRSV ORF1b的氨基酸残基1046至1210的氨基酸序列。在本发明一个实施例中，序列识别号:13的C端氨基酸为丙氨酸(alanine)。

[0049] 上述及其他方面将藉由以下优选实施例的说明参照附图加以陈明。附图绘示本发明的一种或多种实施例，连同书面描述，用以解说本发明的原理。在可能的情况下，所有附图使用相同的附图标记指代一个实施例中相同或类似的组件。

附图说明

[0050] 图1A为一概要图，显示全长绿脓杆菌外毒素A(PE)及PE部分片段。

[0051] 图1B-C显示载体图谱。

[0052] 图1D为一概要图，显示用于制备疫苗组合物PRRSFREE的四种不同质粒。疫苗组合物PRRSFREE包含四种不同单独PE融合蛋白。PRRSFREE疫苗组合物中的个别PE融合蛋白包含PE(ΔIII)片段(PE_{407})、单一抗原部分(称为M、P、R或D)，以及内质网滞留序列(K3)。“D”或“DGD”代表来自PRRSV核蛋白ORF7的抗原。“R”或“RSAB”代表PRRSV ORF6/膜蛋白与ORF5/主要包膜蛋白的融合抗原，两者间并无桥梁/连结序列。“M”或“M12”代表来自PRRSV ORF1b的抗原，其为PRRSV非结构蛋白NSP 10与NSP 11的人造融合抗原。“P”或“PQAB”代表PRRSV ORF6/膜蛋白与ORF5/主要包膜蛋白的融合抗原，两者间并无桥梁/连结序列。“PE(ΔIII)”代表PE片段，不带细胞毒素结构域III。“PE_{407}”代表从氨基酸1至407的绿脓杆菌外毒素A(PE)多肽。

[0053] 图1E为一概要图，显示用于制备PE融合蛋白的质粒，该PE融合蛋白称为PE-DRMP-NESK或PRRSFREE四合一。PE-DRMP-NESK融合蛋白包含PE(ΔIII)片段(PE_{313})、具有四个抗原部分(称为DRMP)的单一融合多肽、核输出信号(NES)以及内质网滞留序列(K)。

[0054] 图1F为一概要图，显示编码融合蛋白的质粒，该融合蛋白包含PE(ΔIII)片段(PE_{313})、单一抗原部分(称为PCV2)、核输出信号(NES)以及内质滞留序列(K)。

[0055] 图2A为一图表，显示使用PBS或疫苗组合物PRRSFREE四合一或PRRSFREE免疫的小鼠的抗原特异性细胞介导免疫(CMI)反应。

[0056] 图2B为一图表，显示使用PBS或疫苗组合物PRRSFREE四合一或PRRSFREE免疫的小鼠的抗原特异性抗体(IgG)反应。

[0057] 图3A为一图表，显示使用PBS或不同PRRS/PCV2混合疫苗免疫的小鼠的PRRSFREE抗原特异性CMI反应。

[0058] 图3B为一图表，显示使用PBS或不同PRRS/PCV2ORF2混合疫苗免疫的小鼠的PCV2ORF2抗原特异性CMI反应。

[0059] 图4A为一图表，显示使用PBS或不同PRRS/PCV2混合疫苗免疫的小鼠的PRRSFREE抗原特异性抗体(IgG)反应。

[0060] 图4B为一图表，显示使用PBS或不同PRRS/PCV2混合疫苗免疫的小鼠的PCV2ORF2抗原特异性抗体(IgG)反应。

[0061] 图5为一图表，显示使用以下项目免疫的小鼠的PRRSFREE抗原特异性CMI反应：(1)融合蛋白，包含两种抗原的融合(亦即，在PE_{313}-DR-NESK中抗原D与R的融合)；或(2)两种不同融合蛋白的组合，各融合蛋白包含两种抗原的融合(亦即，在PE_{313}-DR-NESK中抗原D与R的融合，或在PE_{313}-MP-NESK中抗原M与P的融合)；或(3)融合蛋白，包含四种抗原的融合(亦即，在PE_{313}-DRMP-NESK中抗原D、R、M与P的融合)。

[0062] 图6为一概要图，显示用于对猪进行免疫接种以抗PRRSV感染的各种融合蛋白。

[0063] 图7为一图表，显示在不同PRRSV抗原刺激后的免疫猪中的PSMC所分泌出的IFN-γ表达量。

具体实施方式

[0064] 为更详述本发明，在此提供下列实例，其仅属说明性质，且本领域的技术人员应了解可进行诸多修改变化。现在详述本发明的各种实施例。参照附图，各图中以类似附图标记指代类似的组件。如在本说明书及权利要求书中所使用，除非上下文另有明确的不同指定，否则“一”及“该”的意义包括复数指称。此外，如在本说明书及下文的权利要求书中所使用，除非上下文另有明确的不同指定，否则“在其中”的意义包括“在其中”及“在其上”。再者，说明书中所用的标题或副标题为便利读者，不应对本发明的范围构成影响。此外，本说明书中所涉及的部分术语采用如下定义。

[0065] 定义

[0066] 本说明书中所用术语一般而言具有其在本领域中与本发明语境相关范围及所用处的特定语境范围内通常具有的意义。在此处或本说明书中他处针对用于本发明的特定术语加以陈述，提供额外指引以利于阅读本发明的实行者了解。为便于阅读，某些术语可能以强调方式表达，例如使用斜体字和/或引号。强调方式的使用并不对于术语的范围及意义构成影响；在同一语境中，术语不论是否以强调方式表达，其范围及意义均相同。应知一事物可以多种方式表述。因此，在此讨论的一项或多项术语可使用替代语言及同义字，且一术语不论是否在此阐述或讨论，均不增减其重要性。在此提供某些术语的同义字。使用一种或多种同义字并不排除其他同义字的使用。在本说明书中任何部分的实例使用如果包括任何在此讨论术语的范例，仅属说明性质，且绝不对本发明或任何例示术语的范围及意义构成限制。同样，本发明并不限于本说明书中所提供的各种实施例。

[0067] 除非另有不同定义，在此使用所有技术及科学术语与在本领域的技术人员共同了解的意义相同。若有抵触，应以本文件及其所提供的定义为准。

[0068] 术语“抗原呈递细胞(an antigen-presenting cell,APC)或辅助细胞”意指一种细胞，可在其表面展现与主要组织兼容性复合物(major histocompatibility complexes,MHC)复合的外来抗原。T细胞可使用其T细胞受体(T-cell receptors,TCR)辨识这些复合物。这些细胞处理抗原并将其呈递至T细胞。主要种类的专门抗原呈递细胞为树突细胞(dendritic cells,DC)、巨噬细胞(亦为CD4+且因此亦受HIV感染)、单核细胞以及特定B细胞。

[0069] 术语“抗原呈递细胞(APC)结合域”意指可与抗原呈递细胞(APC)结合的区域(其为多肽)。APC结合域可为多肽，包含与选自以下序列识别号的序列至少为90％相同的氨基酸序列：1及8-11。APC结合域为配体，其可辨识并结合至APC上的受体。

[0070] 分化簇91(Cluster of differentiation 91,CD91)为蛋白质，其在细胞膜中构成受体，且参与受体介导的入胞作用。

[0071] 术语“PE_t”意指转位肽(translocation peptide)或转位结构域(translocation domain)，长度为34-112个氨基酸残基。PE_t可包含与序列识别号：2-4及6至少为90％相同的氨基酸序列。例如，PE_t的氨基酸序列可为PE的a.a.280-a.a.313(序列识别号：4)、a.a.268-a.a.313(序列识别号：3)、a.a.253-a.a.313(序列识别号：2)或a.a.253-a.a.364(序列识别号：6)片段。亦即，PE_t的氨基酸序列可包含PE结构域II(a.a.253至a.a.364；序列识别号：6)的任何区域，只要其包含a.a.280-a.a.313(序列识别号：4)的必要序列(亦即必要片段)即可。

[0072] PE_{407}(序列识别号:7)在前案专利(US 7,335,361B2)中称为PE(ΔIII)。

[0073] 术语“最小转位肽(minimum translocation peptide)”意指$PE_{253-313}$(序列识别号:2),其可将抗原转位入目标细胞的细胞质。

[0074] 术语“内质网(endoplasmic reticulum,ER)滞留序列”意指肽,其功能为协助抗原从细胞质转位入内质网,并将抗原留置于内质网腔中。内质网滞留序列包含Lys Asp Glu Leu(KDEL;序列识别号:15)的序列或RDEL。内质网滞留序列可包含序列KDEL、RDEL、KDELKDELKDEL(K3;序列识别号:16)、KKDLRDELKDEL(K3;序列识别号:17)、KKDELRDELKDEL(K3;序列识别号:18)或KKDELRVELKDEL(K3;序列识别号:19)。

[0075] 核输出信号(nuclear export signal,NES)意指在蛋白质中的4个疏水性残基的短氨基酸序列,其运用核输送作用使之经由核孔复合物从细胞核输出至细胞质。NES由外运子(exportins)辨识及固定。最常见的疏水性残基间隔为$L_{xx}KL_{xx}L_xL_x$(序列识别号:13),其中“L”为亮氨酸(leucine),“K”为赖氨酸(lysine),且“x”为任何自然产生的氨基酸。例如,人造NES可包含序列Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Ala(LQKKLEELELA;序列识别号:14)。

[0076] 术语“NESK”意指NES与ER滞留信号的融合肽(亦即NES融合于ER滞留信号)。其为人造肽,具有核输出信号(NES)及ER滞留序列的功能。因此,其可从细胞核经由核孔复合物输出抗原至细胞质,并协助抗原从细胞质转位至ER,再将抗原留置于ER腔中。例如,NESK的氨基酸序列可为LQKKLEELELAKDEL(序列识别号:12)。

[0077] 亚单位疫苗(Subunit vaccines)为仅使用致病病毒一部分的疫苗。此策略最常用于病毒为疾病成因的情况。该造成疾病的部分为蛋白质,亦即所谓的抗原。

[0078] 抗原(antigen)可为致病蛋白、多肽或肽,其可能造成因病原体产生的疾病,或能够诱发宿主体内的免疫反应。抗原可为融合抗原,由选自一种或多种致病蛋白的两种或更多种抗原融合而成。例如,PRRSV ORF6片段与ORF5片段的融合抗原,或PRRSV与PCV2病原体抗原的融合。

[0079] 表位(epitope)为抗原的一部分。保护性表位(protective epitope)代表当表位与抗体结合时,其可帮助抗体发挥功能而非妨碍抗体。

[0080] 中和表位(neutralizing or neutralization epitopes)的存在为预防性疫苗的结构基础。中和表位是病毒细胞连接/进入的关键。

[0081] 如本文所用,“猪生殖与呼吸综合症病毒(porcine reproductive and respiratory syndrome virus,PRRSV)ORF7抗原”为选自PRRSV ORF7的一部分且包含保护性表位的肽。

[0082] 如本文所用,“PRRSV ORF1b抗原”为选自PRRSV ORF1b的一部分且包含保护性表位的肽。

[0083] 如本文所用,“PRRSV ORF6抗原”为选自PRRSV ORF6的一部分且包含保护性表位的肽。

[0084] 如本文所用,“PRRSV ORF5抗原”为选自PRRSV ORF5的一部分且包含保护性表位的肽。

[0085] 术语“PRRSFREE”意指疫苗组合物,其包含四个融合蛋白PE_{407}-M-K3、PE_{407}-P-K3、PE_{407}-R-K3以及PE_{407}-D-K3。

[0086] 术语“M12”及“M”可互换使用。“M12”如本文所用，意指融合抗原，其为PRRSV NSP 10(C端结构域序列)与NSP 11(N端结构域序列)的融合。

[0087] 术语“PQAB”及“P”可互换使用。“P”如本文所用，意指融合抗原，其为PRRSV ORF6的N端部分与ORF5的N端部分的融合，ORF6与ORF5序列之间并无桥梁/连结序列。

[0088] 术语“RSAB”及“R”可互换使用。“R”如本文所用，意指融合抗原，其为PRRSV ORF6的N端部分与ORF5的N端部分的融合，ORF6与ORF5序列之间并无桥梁/连结序列。

[0089] 术语“DGD”及“D”可互换使用。“D”如本文所用，意指抗原，其包含PRRSV ORF7的C端部分的两个重复序列。

[0090] 术语“治疗(treating or treatment)”意指对罹有癌症，或受感染，或展现这些疾病的症状或倾向，而需治疗的个体施以有效量的融合蛋白，以期治疗、缓和、减轻、医治、改善或预防疾病、其症状或其倾向。此个体可由健康照护专业人士基于任何适当诊断方法的结果加以辨识。

[0091] 术语“有效量(effective amount)”意指活性化合物足以对受治疗个体产生治疗效果的需要量。有效剂量可能依据施药路径、赋形剂的使用及与其他治疗方式共享等因素而变化，如本领域的技术人员所知的那些。

[0092] 实施例

[0093] 在不限制本发明范围的前提下，以下提供本发明实施例的范例仪器、装置、方法及其相关结果。标题或副标题的使用仅为便利读者，而不应构成对于本发明范围的限制。此外，以下亦提及并揭露某些理论。然而，只要本发明可依据本发明实施而不拘于任何特定理论或行动方案，则不论这些理论正确与否，均不应构成对于本发明范围的限制。

[0094] 方法

[0095] 融合抗原DRMP及MDPR的合成

[0096] 将编码融合抗原DRMP(序列识别号:52)、MDPR(序列识别号:53)及PCV2ORF2抗原(序列识别号:20)的DNA序列分别合成并进一步克隆入质粒pTAC-2-PE_{313}-NESK或pTAC-2-RAP1-$PEt_{268-313}$-K3。所有合成的序列均经大肠杆菌生长优化。在PCR中使用各自正向及反向引物以提供DRMP或MDPR DNA扩增。扩增后的DNA片段经EcoRI及XhoI消化，而后接合至(ligated into)指定载体中。融合蛋白PE_{313}-PCV2-NESK亦以类似方式克隆。

[0097] 表1显示用于克隆入质粒的正向及反向引物序列。粗体字表示EcoRI剪切位点;斜体字表示SalI剪切位点;斜体加粗表示XhoI剪切位点;加注下划线表示抗原序列。

[0098] 表1

[0099]

质粒	正向引物	反向引物
克隆 DRMP 至 pTAC-2-PE_{313}-NESK	**gaattc***gtcgac*caccactttaccccgagt (序列识别号：42)	***ctcgag***agcccagtcgaatttgttagccag (序列识别号：43)
克隆 MDPR 至 pTAC-2-PE_{313}-NESK	**gaattc**aataacaaagaatgcacggttgct (序列识别号：44)	***ctcgag***agcccagtcaaagtggttagacag (序列识别号：45)
克隆 DRMP 至 pTAC-2-RAP1-$PEt_{268-313}$-K3	**gaattc**caccactttaccccgagtgagcgt (序列识别号：46)	***ctcgag***agcccagtcgaatttgttagccag (序列识别号：47)
克隆 MDPR 至 pTAC-2-RAP1-$PEt_{268-313}$-K3	**gaattc**aataacaaagaatgcacggttgct (序列识别号：48)	***ctcgag***agcccagtcaaagtggttagacag (序列识别号：49)
克隆 PCV2 ORF2 至 pTAC-2-PE_{313}-NESK	**gaattc**aatggcattttca (序列识别号：50)	***ctcgag***ggggttcaaggg (序列识别号：51)

[0100] 实施例1

[0101] 表达载体的构建

[0102] 图1A显示PE包含三个结构域(I、II及III)。PE_{407}为PE中从a.a.1至a.a407的区域。PE_{407}并不包含细胞毒素结构域III，因此包含结构域I及II。PE_{313}为PE中从a.a.1至a.a.313的区域。因此，PE_{313}仅包含PE的结构域Ia及结构域II的一部份N端区域。

[0103] 图1B-C显示表达载体的构建，其各包含抗原呈递细胞(APC)结合域、转位肽、抗原、具有(下栏)或不具(上栏)核输出信号(NES)，以及内质网(ER)滞留序列(上栏为K3或下栏为K)，该内质网滞留序列位于融合蛋白的C端。质粒pTac-2-PE_{313}-NESK、pTac-2-PE_{407}-K3、pTac-2-RAP1-$PE_{268-313}$-NESK及pTac-2-RAP1-$PE_{268-313}$-K3的产生方式如下：以PCR方法合成$^{NdeI}PE_{313}-^{(EcoRI,XhoI)}$-$NESK^{XhoI}$、$^{NdeI}PE_{407}-^{(EcoRI,XhoI)}$-$K3^{XhoI}$、^{NdeI}RAP1-$^{(EcoRI)}$-$PE_{268-313}-^{(EcoRI,XhoI)}$-$NESK^{XhoI}$及^{NdeI}RAP1-$^{(EcoRI)}$-$PE_{268-313}-^{(EcoRI,XhoI)}$-$K3^{XhoI}$片段，而后以抗卡那霉素基因(kanamycin resistance gene)接合至pUC18骨架以取得各自的质粒。

[0104] 之后可将编码抗原或特定病原体融合抗原的目标DNA嵌入上述质粒以产生表达载体，用以表达融合蛋白。例如，合成编码猪圆环病毒2型(PCV2)ORF2(序列识别号:20)抗原的DNA片段并将其嵌入质粒pTac-2-PE_{313}-NESK以产生表达载体PE_{313}-PCV2-NESK(图1F)。

[0105] 合成以下目标DNA片段：

[0106] (i)目标DNA，其编码包含PRRSV ORF7的C端部分两个重复序列的抗原。此抗原称为“DGD”或“D”。

[0107] (ii)目标DNA，其编码由PRRSV NSP 10(C端结构域序列)与NSP 11(N端结构域序列)构成的融合抗原。此抗原称为“M12”或“M”。

[0108] (iii)目标DNA，其编码由PRRSV ORF6的N端部分与ORF5的N端部分构成的融合抗原，序列ORF6与ORF5序列之间并无桥梁/连结。此抗原称为“RSAB”或“R”。

[0109] (iv)目标DNA，其编码由PRRSV ORF6的N端部分与ORF5的N端部分构成的融合抗原，序列ORF6与ORF5序列之间并无桥梁/连结。此抗原称为“PQAB”或“P”。

[0110] 将上述目标DNA片段嵌入图1B上栏所示的质粒，分别产生融合蛋白PE_{407}-M-K3、PE_{407}-P-K3、PE_{407}-R-K3及PE_{407}-D-K3(图1D)。

[0111] 合成目标DNA片段，其编码包含上述所有四种抗原D、R、M及P(例如DRMP、MDPR等等)的融合抗原，并将其嵌入质粒pTac-2-PE_{313}-NESK以产生表达载体，用以表达融合蛋白PE-DRMP-NESK(图1E)，此融合蛋白亦称为“PRRSFREE四合一”。

[0112] 实施例2

[0113] 蛋白表达

[0114] 将含有融合蛋白表达质粒的大肠杆菌BL21细胞分别在含有25ppm卡那霉素(kanamycin)的Luria Bertani培养基中培养，于37℃培养。当培养物达到初期指数期时(A600＝0.1至0.4)，将异丙基-β-D-硫代半乳糖苷(isopropyl-1-thio-β-D-galactopyranoside,IPTG)加入，最终浓度为0.5至2mM，以诱发反应。诱发后四小时收获细胞并立即储存于-70℃。如先前所述，藉由使用尿素萃取来纯化融合蛋白(Liao et al.,1995.Appl.Microbiol.Biotechnol.43:498-507)，而后利用50倍量的TNE缓冲液(50mM Tris、50mM NaCl及1mM EDTA)以透析方法重折叠，于4℃温度下过夜。使用Bradford蛋白检验试剂盒(Pierce)对重折叠的蛋白进行SDS-PAGE分析及定量分析。结果显示多数重折叠的蛋白在非还原条件下为单体，显示融合蛋白可轻易重折叠且不会聚集。

[0115] 实施例3

[0116] PRRSV亚单位疫苗的免疫原性分析(immunogenicity assay)

[0117] 以s.c.注射方式对小鼠接种200μl PRRSV亚单位疫苗，其含有每剂30μg的PRRSFREE四合一或PRRSFREE及ISA206佐剂，每周一次，为期两周。对照组(安慰剂)注射PBS。

[0118] 所有小鼠于最后一次免疫接种后14天牺牲，收获其脾脏。分离出脾细胞并置入96孔盘(10^5细胞/100μl/孔)，加入或不加入刺激性重组抗原蛋白，并在37℃的温度下培养72小时。依据免疫接种所用疫苗，对应的刺激性重组抗原蛋白为PRRSFREE抗原、PRRSFREE-四合一嵌合性融合抗原或PCV2 ORF2抗原，以检测抗原特异性细胞介导的免疫反应。收集细胞培养上清液，并使用IFN-γ小鼠抗体对(Mouse Antibody Pair)(Invitrogen)测定上清液中的干扰素-γ(IFN-γ)。

[0119] 依据免疫接种所用疫苗，将PRRSFREE抗原或PRRSFREE四合一融合抗原或PCV2ORF2抗原涂布于ELISA中以检测体液免疫反应。涂布后，将培养皿清洗并封闭，接着加入稀释后的小鼠血清。而后清洗培养皿，以HRP结合的二级抗体杂交，继而加入TMB基质。反应停止后，以ELISA读取器检测结果。

[0120] 实施例4

[0121] 细胞介导的免疫反应(Cell-mediated immune response,CMI)以及体液免疫反应

[0122] 图2A显示接种组的IFN-γ浓度高于对照组，证明接种诱发CMI反应。此外，PRRSFREE四合一疫苗使用组的IFN-γ浓度高于PRRSFREE治疗组。意料外的结果证明由单一融合抗原构成的PRRSFREE四合一疫苗较由四种抗原组成的PRRSFREE疫苗能够诱发更强的CMI反应。

[0123] 图2B显示疫苗免疫组较对照组具有更高的抗原特异性抗体。小鼠以PRRSFREE四合一疫苗接种后，具有较注射PRRSFREE疫苗组别更高的抗体滴度。结果显示PRRSFREE四合一可较PRRSFREE疫苗诱发更高的体液免疫反应。

[0124] 图2A-B的数据显示PRRSFREE四合一，包含由D、M、P及R抗原融合而成的单一融合抗原所构成的融合蛋白，较包含四种分别位于各自融合蛋白的分离单独抗原(亦即四种抗原D、M、P、R未融合)的PRRSFREE疫苗能够引发更高的细胞及体液免疫反应。

[0125] 实施例5

[0126] 具有猪圆环病毒2型(PCV2)ORF2亚单位疫苗的组合疫苗

[0127] 依据上述的免疫接种时程，以PBS、PRRSFREE四合一加PE-PCV2-NESK，或PRRSFREE加PE-PCV2-NESK混合疫苗对小鼠接种。PRRSFREE四合一加PE-PCV2-NESK混合疫苗包含PE-DRMP-NESK(图1E)以及PE-PCV2-NESK(图1F)融合蛋白。PRRSFREE加PE-PCV2-NESK混合疫苗包含5种分离融合蛋白：(1)PE-DGD-K3、PE-M12-K3、PE-PQAB-K3、PE-RSAB-K3(图1D)及PE-PCV2-NESK(图1F)。

[0128] 实施例6

[0129] 具有PCV2 ORF2亚单位疫苗的组合疫苗

[0130] 图3A显示PRRSV抗原特异性(PRRSFREE四合一融合抗原及PRRSFREE抗原)CMI反应，以及图3B显示PCV2-ORF2抗原特异性CMI反应。数据指出以PRRSFREE四合一融合抗原与PCV2ORF2亚单位疫苗组合进行免疫的小鼠较以PRRSFREE(4种分离抗原)与PCV2ORF2亚单位疫苗组合进行免疫的小鼠展现更强的CMI反应。

[0131] 图4A显示PRRSV抗原特异性抗体反应。使用ELISA方法测量抗原特异性抗体滴度。对于以PE-DRMP-NESK与PE-PCV2-NESK(亦即两种融合蛋白)组合治疗的组别，使用融合抗原DRMP测量抗原特异性抗体滴度。对于以PRRSFREE与PE-PCV2-NESK(亦即5种融合蛋白)组合治疗的组别，使用四种抗原D、R、M及P测量抗原特异性抗体滴度。数据指出使用PRRSFREE四合一融合抗原与PCV2ORF2亚单位疫苗组合进行免疫的小鼠较以PRRSFREE(4种分离抗原)与PCV2ORF2亚单位疫苗组合进行免疫的小鼠展现更强的PRRSFREE四合一融合抗原特异性体液反应(图4A)。

[0132] 图4B显示PCV2-ORF2抗原特异性抗体反应。意外发现以PRRSFREE(4种分离抗原)与PCV2ORF2亚单位疫苗(PE-PCV2-NESK)组合免疫的小鼠较以PRRSFREE四合一融合抗原(PE-DRMP-NESK)与PCV2ORF2亚单位疫苗(PE-PCV2-NESK)组合免疫的小鼠具有更高的PCV2特异性抗体滴度。结果显示两种PRRSV/PCV2混合疫苗之间存在有不同的PRRSV抗原特异性及PCV2抗原特异性的体液免疫反应。

[0133] 显然两种方案皆可有效诱发CMI及体液免疫反应。PRRSV/PCV2混合疫苗包含2种融合蛋白(PE-DRMP-NESK与PE-PCV2-NESK)，在四种受测免疫反应中的三者显示较佳效率。此研究证明由PRRSV嵌合抗原与PCV2ORF2抗原构成的PRRSV/PCV2混合疫苗优于由五种个别抗原所组成的疫苗。然而两种方案皆可在动物体内诱发免疫反应。

[0134] 实施例7

[0135] 两种抗原的融合与四种抗原的融合

[0136] 三组六周龄雌性C57BL/6小鼠(每组3只小鼠)以皮下注射方式施予(1)15μg的PE-DR-NESK蛋白、(2)15μg的PE-DR-NESK与15μg的PE-MP-NESK蛋白的组合，或(3)30μg的PE-DRMP-NESK，皆配于200μl的50％ISA206中，以周间间隔(weekly intervals)施打三次。最后一次免疫接种后一周牺牲小鼠，取得脾脏细胞。将脾脏细胞以4种PRRSV抗原(M12、DgD、PQAB及RSAB，各2.5μg/ml)刺激72小时，并使用ELISA试剂盒检测各组无细胞上清液中的IFN-γ。

图5显示以PE-DRMP-NESK免疫的小鼠在三组中展现最强的CMI反应。

[0137] 实施例8

[0138] 猪的细胞介导的免疫力(Cell-mediated immunity)

[0139] 以下列疫苗之一对五周龄的SPF猪(每组2-4头猪只)实施肌内注射:(1)PRRSFREE、(2)PE-DRMP-NESK、(3)PE-MDPR-NESK、(4)RAP1-$PE_{268-313}$-DRMP-K3、(5)RAP1-$PE_{268-313}$-MDPR-K3,皆配于2mL的50％ISA206中,或(6)PBS作为安慰剂,以周间间隔施打两次。图6显示上述疫苗的设计。每次注射所含抗原为300μg,其配于2mL的50％ISA206中。最后一次免疫处理的三周后采集接种后猪的外周血液单核细胞(Peripheral blood mononuclear cells,PBMCs)。依据用于免疫的疫苗,PBMC的刺激是使用PRRSFREE抗原(M12、DgD、PQAB及RSAB,各2.5ug/ml)、PE-DRMP-NESK、PE-MDPR-NESK、RAP1-$PE_{268-313}$-DRMP-K3或RAP1-$PE_{268-313}$-MDPR-K3,时间为72小时,而后使用ELISA试剂盒检测各组无细胞上清液中的IFN-γ。图7显示接种后猪的PBMC经刺激后所分泌IFN-γ。结果显示包含融合抗原的疫苗较安慰剂组更能够诱发IFN-γ分泌。

[0140] 实施例9

[0141] 猪病毒血症研究(Viremia studies)

[0142] 以下列疫苗之一对五周龄的SPF猪(每组2-4头猪)实施肌内注射:(1)PRRSFREE、(2)PE-DRMP-NESK、(3)PE-MDPR-NESK、(4)RAP1-$PE_{268-313}$-DRMP-K3、(5)RAP1-$PE_{268-313}$-MDPR-K3,皆配于2mL的50％ISA206中,或(6)PBS作为安慰剂,以周间间隔施打二次。每次注射所含抗原为300μg,其配于2mL的50％ISA206中。最后一次免疫处理的三周后对接种后的猪经鼻腔施予2x 10^5TCID50的PRRSV。每周收集血液样本。从血清中提取病毒RNA并使用单一步骤SyBR Green实时PCR定量,以判定病毒血症的程度。实验结果显示包含融合抗原的疫苗能够降低病毒量。

[0143] 表2显示用于制作各种融合蛋白的肽序列识别号。

[0144] 表2

[0145]

组成	序列识别号：	长度
最小绿脓杆菌外毒素 A(PE)结合域 Ia (APC 结合域，PE 的 a.a.1- a.a.252)	1	252
$PE_{253-313}$ (转位结构域)	2	61
$PE_{268-313}$ (转位结构域)*	3	46
PE_t Core (PE 转位结构域核心；PE 的 a.a. 280- a.a. 313)	4	34
PE_{313} (PE 的 a.a. 1- a.a. 313)	5	313
$PE_{253-364}$ (转位结构域)	6	112
PE_{407} (PE 的 a.a. 1- a.a. 407)	7	407
RAP1 Minimum (RAP1 的第三区)	8	104
A2M Minimum	9	153
HIV-Tat Minimum	10	24
HSPs Minimum	11	641
NESK 为 LQKKLEELELAKDEL **	12	15
NES 共有序列为 $L_{XX}KL_{XX}L_XL_X$，其中“L”为亮氨酸，“K”为赖氨酸且“$_X$”为任何自然产生的氨基酸。	13	11
NES 为 LQKKLEELELA	14	11
KDEL (K)	15	4
KDELKDELKDEL (K3)	16	12
KKDLRDELKDEL (K3)	17	12
KKDELRDELKDEL (K3)	18	13
KKDELRVELKDEL (K3)	19	13

	PCV2 ORF2 (截短猪圆环病毒 2 型 ORF2；aa 42 -aa 233)	20	192
	全长 PE (绿脓杆菌外毒素 A)	21	613
	DGD (ORF7 抗原，有串联重复序列 D)	22	220
	D (ORF7 抗原，无串联重复序列 D) RHHFTPSERQLCLSSIQTAFNQGAGTCILSDSGRISYTVEF SLPTHHTVRLIRVTAPPSA	23	60
	R (RSAB)	24	62
	M (M12)***	25	165
	P (PQAB)****	26	58
	PE_{313}-DRMP-NESK (或 PE-DRMP-NESK)	27	841
	PE_{313}-MDPR-NESK (或 PE-MPDR-NESK)	28	746
	RAP1-$PE_{268-313}$-DRMP-K3	29	677
	RAP1-$PE_{268-313}$-MDPR-K3	30	584
	PE_{313}-PCV2-NESK (或 PE-PCV2-NESK)	31	525
	PE_{1-267} (PE 结合域)	32	267
[0146]	替代性 D (ORF7 抗原，无串联重复序列 D) HHFTPSERQLCLSSIQTAFNQGAGTCILSDSGRISYTVEFS LPTHHTVRLIRVTAPPSA	33	59
	ORF6 的 N 端部分[从台湾所得到的 PRRSV 分离株] GSSLDDFCYDSTAPQKVLLAFSITY	34	25
	ORF5 的 N 端部分[从台湾所得到的 PRRSV 分离株] ASNDSSSHLQLIYNLTLCELNGTDWLANKFDWA	35	33
	ORF6 的 N 端部分 MGSLDDFCNDSTAAQKLVLAFSITYTPI	36	28
	ORF5 的 N 端部分 FVAGGSSSTYQYIYNLTICELNGTDWLSNHFDWA	37	34
	PRRSV ORF5， 1 型(欧洲) PRRSV 株 MRCSHKLGRFLTPHSCFWWLFLLCTGLSWS**FVAGGSSS TYQYIYNLTICELNGTDWLSNHFDWA**VETFVL YPVATHILSLGFLTTSHFFDALGLGAVSTIGFVGGRYVLS SVYGACAFAAFVCFVIRAVKNCMAFRYAHT	38	200

[0147]

RFTNFIVDDRGRIHRWKSPIVVEKLGKAEVGGDLVTIKH VVLEGVKAQPLTRTSAEQWEA		
PRRSV ORF6， 1 型(欧洲) PRRSV 株 **MGSLDDFCNDSTAAQKLVLAFSITYTPI**MIYALKVSRG RLLGLLHILIFLNCSFTFGYMTYVRFQSTNRV ALTLGAVVALLWGVYSFTESWKFVTSRCRLCCLGRRYI LAPAHHVESAAGLHSIPASGNRAYAVRKPGLT SVNGTLVPGLRSLVLGGKRAVKRGVVNLVKYGR	39	173
PRRSV ORF5， 2 型(北美) PRRSV 株 MLGKCLTAGCCSRLLFLWCTVPSCFVALVG**ASNSSSSYS QLIYNLTLCELNGTDWLANKFDWA**VETFVIF PVLTHIVSYGALTTSHFLDTVGLITVSTAGFYHGRYVLSS IYATCALAALICFVIRLAKNCMSWRYSCTR YTNFLLDTKGRIYRWRSPVIIEKGGKVEVEGHLIDLKRV VLDGSAATPVTKISAEQWGRP	40	200
PRRSV ORF6： 2 型(北美) PRRSV 株 M**GSSLDDFCHDSTAPQKVILAFSITY**TPVMIYALKVSR GRLLGLLHLLIFLNCAFTFGYMTFVHFQSTNR VALTMGAVVALLWGVYSAIETWRFITSRCRLCLLGRKYI LAPAHHVESAAGFHPIAASDNHAFVVRRPGS TTVNGTLVPGLKSLVLGGRKAVKQGVVNLVKYAK	41	174

[0148] *:$PE_{268-313}$为全长PE的a.a.268-a.a.313;PE_{313}为全长PE的a.a.1-a.a.313;PE_{407}为全长PE的a.a.1-a.a.407。

[0149] **:粗体字代表人造核输出信号的氨基酸序列;加注下划线字体代表内质网滞留信号的氨基酸序列。

[0150] ***:M(M12)为融合多肽,经融合PRRSV NSP 10(C端结构域序列)与NSP 11(N端结构域序列)制备而成。亦即,所述多肽取自ORF1b非结构蛋白NSP 10的C端部分及NSP 11的N端部分。

[0151] ****:P(PQAB)为多肽,经融合PRRSV ORF6a.a.2-a.a.26与ORF5aa 31-aa 63制备而成。参阅美国专利第7465455号。以一般字体表示的序列取自PRRSV ORF6/基质蛋白序列,且以粗体字表示的序列取自PRRSV ORF 5序列。由PRRSV的ORF5编

[0152] 码的主要包膜蛋白(GP5)在诱发病毒中和(VN)抗体及不同 PRRSV 株间相互保护方面扮演关键角色。由于不同株间的序列变化，在此揭露的序列仅为说明的用途。

[0153] 本发明并不限于所述特定形式,且所有不脱离本发明精神的范畴的修改均应属于所附权利要求书所定义的范畴。实施例及实例的选择与描述为解说本发明的原理及其实务应用,以利于其他本领域的技术人员运用本发明、各种实施例连同各种适合其实务应用的

修改。本领域的技术人员应可思及不脱离本发明精神及范围的替代实施例。本发明引用及讨论的引用文件可能包括专利、专利申请及各种公开文献。这些引用文件的引用和/或讨论仅为澄清本发明的描述，并非承认任何这些引用文件为本发明的“前案”。所有在本说明书中引述及讨论的引用文件均以其引用而整体合并于此，如同各引用文件个别合并于此。

[0001] 序列表

[0002] <110> 瑞宝基因股份有限公司

[0003] <120> 用于猪生殖与呼吸综合症及猪圆环病毒相关疾病的疫苗组合物

[0004] <130> 10025-00002

[0005] <150> 62169205

[0006] <151> 2015-06-01

[0007] <160> 53

[0008] <170> PatentIn version 3.5

[0009] <210> 1

[0010] <211> 252

[0011] <212> PRT

[0012] <213> 绿脓杆菌

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[0023] 65 70 75 80

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[0031] 130 135 140

[0032] Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn Glu

[0033] 145 150 155 160

[0034] Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met

[0035] 165 170 175

[0036] Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser

[0037] 180 185 190

[0038] Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr

[0039] 195 200 205

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[0088] 20 25 30

[0089] Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val

[0090] 35 40 45

[0091] Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu

[0092] 50 55 60

[0093] Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu

[0094] 65 70 75 80

[0095] Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser

[0096] 85 90 95

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[0105] Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met

[0106] 165 170 175

[0107] Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser

[0108] 180 185 190

[0109] Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr

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[0111] Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile

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[0113] Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys

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[0117] Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr Phe

[0118] 260 265 270

[0119] Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly

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[0124] 305 310

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[0139] 65 70 75 80

[0140] Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu

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[0157] Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu

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[0159] Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser

[0160] 85 90 95

[0161] Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn

[0162] 100 105 110

[0163] Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His

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[0175] Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile
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[0177] Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys
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[0183] Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly
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[0216] Arg Thr Lys Glu Leu Gly Tyr Thr Val Lys Lys His Leu Gln Asp Leu
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[0235] Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu Glu Arg
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[0243] Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr Asn Ala
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[0269] Gln Gly Asn Arg Thr Thr Pro Ser Tyr Val Ala Phe Thr Asp Thr Glu

[0270] 35 40 45

[0271] Arg Leu Ile Gly Asp Ala Ala Lys Asn Gln Val Ala Leu Asn Pro Gln

[0272] 50 55 60

[0273] Asn Thr Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Lys Phe Gly Asp

[0274] 65 70 75 80

[0275] Pro Val Val Gln Ser Asp Met Lys His Trp Pro Phe Gln Val Ile Asn

[0276] 85 90 95

[0277] Asp Gly Asp Lys Pro Lys Val Gln Val Ser Tyr Lys Gly Glu Thr Lys

[0278] 100 105 110

[0279] Ala Phe Tyr Pro Glu Glu Ile Ser Ser Met Val Leu Thr Lys Met Lys

[0280] 115 120 125

[0281] Glu Ile Ala Glu Ala Tyr Leu Gly Tyr Pro Val Thr Asn Ala Val Ile

[0282] 130 135 140

[0283] Thr Val Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Asp

[0284] 145 150 155 160

[0285] Ala Gly Val Ile Ala Gly Leu Asn Val Leu Arg Ile Ile Asn Glu Pro

[0286] 165 170 175

[0287] Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Arg Thr Gly Lys Gly Glu

[0288] 180 185 190

[0289] Arg Asn Val Leu Ile Phe Asp Leu Gly Gly Gly Thr Phe Asp Val Ser

[0290] 195 200 205

[0291] Ile Leu Thr Ile Asp Asp Gly Ile Phe Glu Val Lys Ala Thr Ala Gly

[0292] 210 215 220

[0293] Asp Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg Leu Val Asn His

[0294] 225 230 235 240

[0295] Phe Val Glu Glu Phe Lys Arg Lys His Lys Lys Asp Ile Ser Gln Asn

[0296] 245 250 255

[0297] Lys Arg Ala Val Arg Arg Leu Arg Thr Ala Cys Glu Arg Ala Lys Arg

[0298] 260 265 270

[0299] Thr Leu Ser Ser Ser Thr Gln Ala Ser Leu Glu Ile Asp Ser Leu Phe

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[0301] Glu Gly Ile Asp Phe Tyr Thr Ser Ile Thr Arg Ala Arg Phe Glu Glu

[0302] 290 295 300

[0303] Leu Cys Ser Asp Leu Phe Arg Ser Thr Leu Glu Pro Val Glu Lys Ala

[0304] 305 310 315 320

[0305] Leu Arg Asp Ala Lys Leu Asp Lys Ala Gln Ile His Asp Leu Val Leu

[0306] 325 330 335

[0307] Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Lys Leu Leu Gln Asp

[0308] 340 345 350

[0309] Phe Phe Asn Gly Arg Asp Leu Asn Lys Ser Ile Asn Pro Asp Glu Ala

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[0311] Val Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Met Gly Asp Lys

[0312] 370 375 380

[0313] Ser Glu Asn Val Gln Asp Leu Leu Leu Leu Asp Val Ala Pro Leu Ser

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[0315] Leu Gly Leu Glu Thr Ala Gly Gly Val Met Thr Ala Leu Ile Lys Arg

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[0317] Asn Ser Thr Ile Pro Thr Lys Gln Thr Gln Ile Phe Thr Thr Tyr Ser

[0318] 420 425 430

[0319] Asp Asn Gln Pro Gly Val Leu Ile Gln Val Tyr Glu Gly Glu Arg Ala

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[0322] 450 455 460

[0323] Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile

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[0325] Asp Ala Asn Gly Ile Leu Asn Val Thr Ala Thr Asp Lys Ser Thr Gly

[0326] 485 490 495

[0327] Lys Ala Asn Lys Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser Lys

[0328] 500 505 510

[0329] Glu Glu Ile Glu Arg Met Val Gln Glu Ala Glu Lys Tyr Lys Ala Glu

[0330] 515 520 525

[0331] Asp Glu Val Gln Arg Glu Arg Val Ser Ala Lys Asn Ala Leu Glu Ser

[0332] 530 535 540

[0333] Tyr Ala Phe Asn Met Lys Ser Ala Val Glu Asp Glu Gly Leu Lys Gly

[0334] 545 550 555 560

[0335] Lys Ile Ser Glu Ala Asp Lys Lys Lys Val Leu Asp Lys Cys Gln Glu

[0336] 565 570 575

[0337] Val Ile Ser Trp Leu Asp Ala Asn Thr Leu Ala Glu Lys Asp Glu Phe

[0338] 580 585 590

[0339] Glu His Lys Arg Lys Glu Leu Glu Gln Val Cys Asn Pro Ile Ile Ser

[0340] 595 600 605

[0341] Gly Leu Tyr Gln Gly Ala Gly Gly Pro Gly Pro Gly Gly Phe Gly Ala

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[0343] Gln Gly Pro Lys Gly Gly Ser Gly Ser Gly Pro Thr Ile Glu Glu Val

[0344] 625 630 635 640

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[0349] <213> 人工序列

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[0351] <223> NESK

[0352] <400> 12

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[0354] 1 5 10 15

[0355] <210> 13

[0356] <211> 11

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[0358] <213> 人工序列

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[0360] <223> NES 共有序列

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[0367] <222> (6)..(7)

[0368] <223> Xaa可以是任何自然产生的氨基酸

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[0371] <222> (9)..(9)

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[0375] <222> (11)..(11)

[0376] <223> Xaa可以是任何自然产生的氨基酸

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[0379] 1 5 10

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[0406] 1 5 10

[0407] <210> 17

[0408] <211> 12

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[0429] <220>

[0430] <223> ER滞留序列

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[0433] 1 5 10

[0434] <210> 20

[0435] <211> 192

[0436] <212> PRT

[0437] <213> 人工序列

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[0443] Lys Arg Thr Thr Val Lys Thr Pro Ser Trp Ala Val Asp Met Met Arg

[0444] 20 25 30

[0445] Phe Asn Ile Asn Asp Phe Leu Pro Pro Gly Gly Gly Ser Asn Pro Arg

[0446] 35 40 45

[0447] Ser Val Pro Phe Glu Tyr Tyr Ser Ile Ser Lys Val Lys Val Glu Phe

[0448] 50 55 60

[0449] Trp Pro Cys Ser Pro Ile Thr Gln Gly Asp Ser Gly Val Gly Ser Ser

[0450] 65 70 75 80

[0451] Ala Val Ile Leu Asp Asp Asn Phe Val Thr Lys Ala Thr Ala Leu Thr

[0452] 85 90 95

[0453] Tyr Asp Pro Tyr Val Asn Tyr Ser Ser Arg His Thr Ile Thr Gln Pro

[0454] 100 105 110

[0455] Phe Ser Tyr His Ser Arg Tyr Phe Thr Pro Lys Pro Val Leu Asp Ser

[0456] 115 120 125

[0457] Gly Gly Gly Ala Ala Ala Pro Asn Asn Lys Arg Asn Gln Leu Trp Leu

[0458] 130 135 140

[0459] Arg Leu Gln Thr Ala Gly Asn Val Asp His Val Gly Leu Gly Thr Ala

[0460] 145 150 155 160

[0461] Phe Glu Asn Ser Ile Tyr Asp Gln Glu Tyr Asn Ile Arg Val Thr Met

[0462] 165 170 175

[0463] Tyr Val Gln Phe Arg Glu Phe Asn Leu Lys Asp Pro Pro Leu Asn Pro

[0464] 180 185 190

[0465] <210> 21

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[0474] Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val

[0475] 35 40 45

[0476] Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu

[0477] 50 55 60

[0478] Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu

[0479] 65 70 75 80

[0480] Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser

[0481] 85 90 95

[0482] Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn

[0483] 100 105 110

[0484] Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His

[0485] 115 120 125

[0486] Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala Lys

[0487] 130 135 140

[0488] Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn Glu

[0489] 145 150 155 160

[0490] Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met

[0491] 165 170 175

[0492] Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser

[0493] 180 185 190

[0494] Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr

[0495] 195 200 205

[0496] Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile

[0497] 210 215 220

[0498] Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys

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[0500] Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser Leu

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[0502] Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr Phe

[0503] 260 265 270

[0504] Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly
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[0508] Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro Gly
[0509] 305 310 315 320
[0510] Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln Ala
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[0524] Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln
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[0526] Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala
[0527] 450 455 460
[0528] Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala Leu Ala Tyr Gly
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[0530] Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly
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[0542] Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro Asp Lys Glu Gln
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[0557] Gln Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Ile Leu Ser Asp Ser
[0558] 20 25 30
[0559] Gly Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr
[0560] 35 40 45
[0561] Val Arg Leu Ile Arg Val Thr Ala Pro Pro Ser Ala Leu Asp Gln Val
[0562] 50 55 60
[0563] Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
[0564] 65 70 75 80
[0565] Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
[0566] 85 90 95
[0567] Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
[0568] 100 105 110
[0569] Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
[0570] 115 120 125
[0571] Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
[0572] 130 135 140
[0573] Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
[0574] 145 150 155 160
[0575] Arg His His Phe Thr Pro Ser Glu Arg Gln Leu Cys Leu Ser Ser Ile
[0576] 165 170 175
[0577] Gln Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Ile Leu Ser Asp Ser
[0578] 180 185 190
[0579] Gly Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr
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[0581] Val Arg Leu Ile Arg Val Thr Ala Pro Pro Ser Ala
[0582] 210 215 220
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[0594] Gly Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr

[0595] 35 40 45

[0596] Val Arg Leu Ile Arg Val Thr Ala Pro Pro Ser Ala

[0597] 50 55 60

[0598] <210> 24

[0599] <211> 62

[0600] <212> PRT

[0601] <213> 人工序列

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[0603] <223> R (RSAB)

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[1080] Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile

[1081] 225 230 235 240

[1082] Lys Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser

[1083] 245 250 255

[1084] Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr

[1085] 260 265 270

[1086] Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys

[1087] 275 280 285

[1088] Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu

[1089] 290 295 300

[1090] Ser Trp Asn Gln Val Asp Gln Val Ile Arg Glu Phe Asn Gly Ile Phe

[1091] 305 310 315 320

[1092] Asn Thr Arg Leu Ser Arg Thr Phe Gly Tyr Thr Ile Lys Arg Thr Thr
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[1096] Asp Phe Leu Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val Pro Phe
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[1099] 370 375 380
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[1111] 465 470 475 480
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[1113] 485 490 495
[1114] Arg Glu Phe Asn Leu Lys Asp Pro Pro Leu Asn Pro Leu Glu Leu Gln
[1115] 500 505 510
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[1127] Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val
[1128] 35 40 45
[1129] Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu
[1130] 50 55 60
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[1132] 65 70 75 80
[1133] Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser

[1134] 85 90 95
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[1137] Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His
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[1151] Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys
[1152] 225 230 235 240
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[1158] <211> 59
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[1160] <213> 人工序列
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[1162] <223> 替代物D (ORF7抗原，无串联重复序列D)
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[1166] Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Ile Leu Ser Asp Ser Gly
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[1168] Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr Val
[1169] 35 40 45
[1170] Arg Leu Ile Arg Val Thr Ala Pro Pro Ser Ala
[1171] 50 55
[1172] <210> 34
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[1175] <213> 人工序列

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[1181] Val Leu Leu Ala Phe Ser Ile Thr Tyr
[1182] 20 25
[1183] <210> 35
[1184] <211> 33
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[1186] <213> 人工序列
[1187] <220>
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[1190] Ala Ser Asn Asp Ser Ser Ser His Leu Gln Leu Ile Tyr Asn Leu Thr
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[1192] Leu Cys Glu Leu Asn Gly Thr Asp Trp Leu Ala Asn Lys Phe Asp Trp
[1193] 20 25 30
[1194] Ala
[1195] <210> 36
[1196] <211> 28
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[1200] <223> ORF6的N端部分
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[1204] Leu Val Leu Ala Phe Ser Ile Thr Tyr Thr Pro Ile
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[1211] <223> ORF5的N端部分
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[1216] 20 25 30
[1217] Trp Ala

[1218] <210> 38

[1219] <211> 200

[1220] <212> PRT

[1221] <213> 猪生殖与呼吸综合症病毒

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[1225] Phe Trp Trp Leu Phe Leu Leu Cys Thr Gly Leu Ser Trp Ser Phe Val

[1226] 20 25 30

[1227] Ala Gly Gly Ser Ser Ser Thr Tyr Gln Tyr Ile Tyr Asn Leu Thr Ile

[1228] 35 40 45

[1229] Cys Glu Leu Asn Gly Thr Asp Trp Leu Ser Asn His Phe Asp Trp Ala

[1230] 50 55 60

[1231] Val Glu Thr Phe Val Leu Tyr Pro Val Ala Thr His Ile Leu Ser Leu

[1232] 65 70 75 80

[1233] Gly Phe Leu Thr Thr Ser His Phe Phe Asp Ala Leu Gly Leu Gly Ala

[1234] 85 90 95

[1235] Val Ser Thr Ile Gly Phe Val Gly Gly Arg Tyr Val Leu Ser Ser Val

[1236] 100 105 110

[1237] Tyr Gly Ala Cys Ala Phe Ala Ala Phe Val Cys Phe Val Ile Arg Ala

[1238] 115 120 125

[1239] Val Lys Asn Cys Met Ala Phe Arg Tyr Ala His Thr Arg Phe Thr Asn

[1240] 130 135 140

[1241] Phe Ile Val Asp Asp Arg Gly Arg Ile His Arg Trp Lys Ser Pro Ile

[1242] 145 150 155 160

[1243] Val Val Glu Lys Leu Gly Lys Ala Glu Val Gly Gly Asp Leu Val Thr

[1244] 165 170 175

[1245] Ile Lys His Val Val Leu Glu Gly Val Lys Ala Gln Pro Leu Thr Arg

[1246] 180 185 190

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[1248] 195 200

[1249] <210> 39

[1250] <211> 173

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[1252] <213> 猪生殖与呼吸综合症病毒

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[1257] 20 25 30

[1258] Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Ile Leu Ile

[1259] 35 40 45

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[1288] 50 55 60
[1289] Glu Thr Phe Val Ile Phe Pro Val Leu Thr His Ile Val Ser Tyr Gly
[1290] 65 70 75 80
[1291] Ala Leu Thr Thr Ser His Phe Leu Asp Thr Val Gly Leu Ile Thr Val
[1292] 85 90 95
[1293] Ser Thr Ala Gly Phe Tyr His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
[1294] 100 105 110
[1295] Ala Thr Cys Ala Leu Ala Ala Leu Ile Cys Phe Val Ile Arg Leu Ala
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[1298] 130 135 140
[1299] Leu Leu Asp Thr Lys Gly Arg Ile Tyr Arg Trp Arg Ser Pro Val Ile
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[1301] Ile Glu Lys Gly Gly Lys Val Glu Val Glu Gly His Leu Ile Asp Leu

[1302] 165 170 175
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[1306] 195 200
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[1310] <213> 猪生殖与呼吸综合症病毒
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[1315] 20 25 30
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[1317] 35 40 45
[1318] Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Val His
[1319] 50 55 60
[1320] Phe Gln Ser Thr Asn Arg Val Ala Leu Thr Met Gly Ala Val Val Ala
[1321] 65 70 75 80
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[1323] 85 90 95
[1324] Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
[1325] 100 105 110
[1326] Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Ser
[1327] 115 120 125
[1328] Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
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[1332] Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
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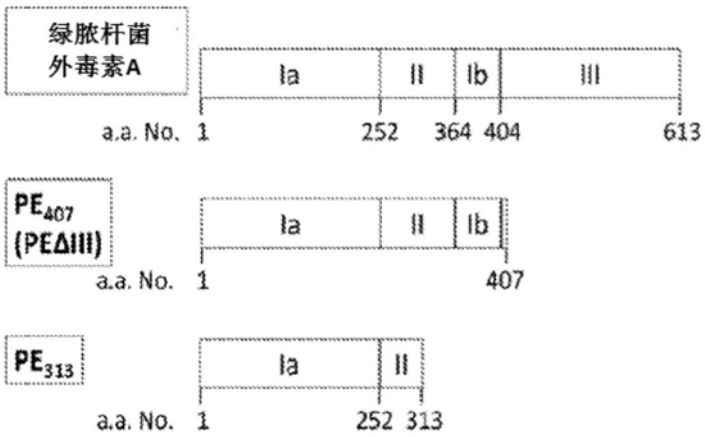
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外毒素A
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II
Ib
III
a.a. No. 1
252
364
404
613
PE407
(PEΔIII)
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Ia
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252
313

图1A

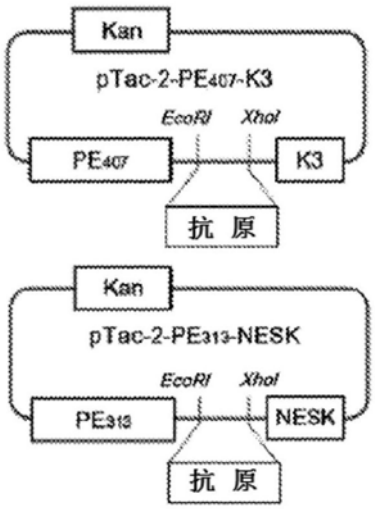
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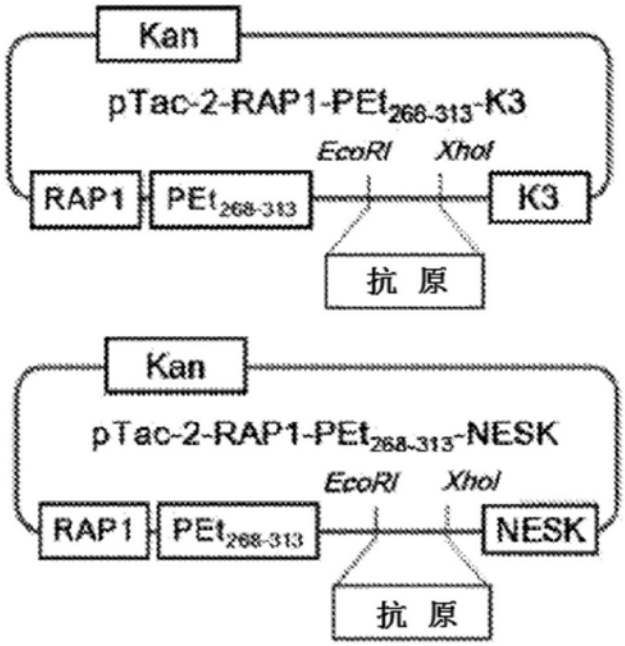
图1B



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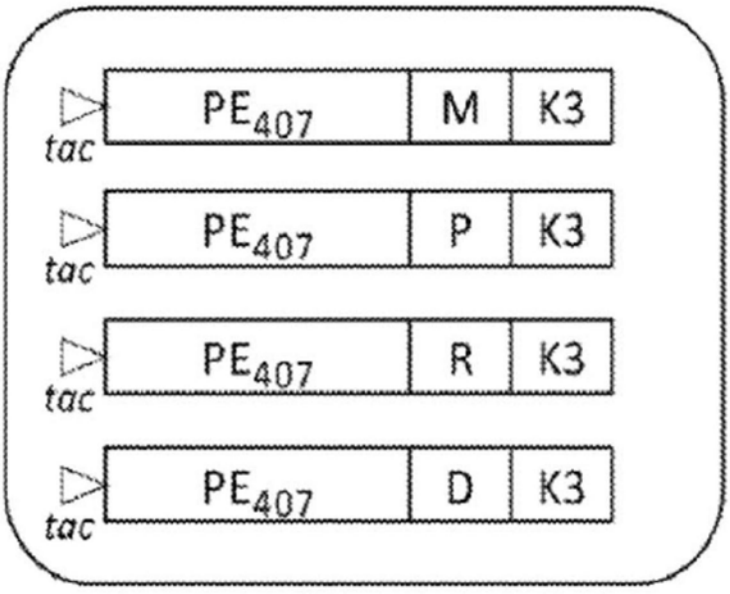
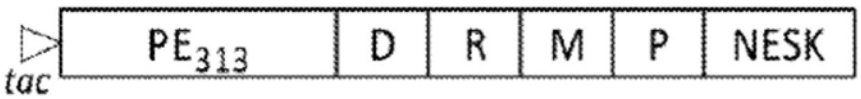


PRRSFREE

图1D



PE-DRMP-NESK
(PRRSFREE四合一)

图1E


tac
PE313
PCV2
NESK
PE-PCV2-NESK

图1F

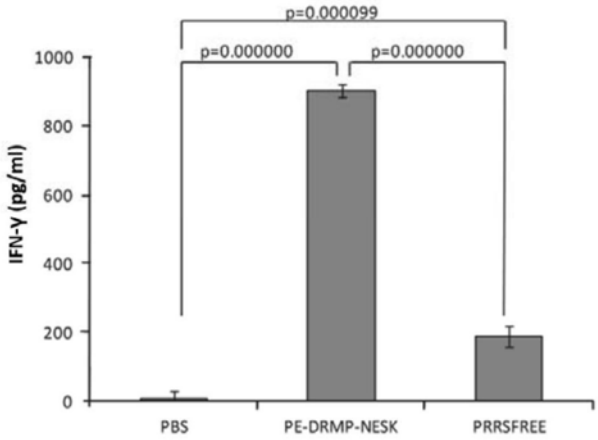

p=0.000099
p=0.000000
p=0.000000
IFN-γ (pg/ml)
1000
800
600
400
200
0
PBS
PE-DRMP-NESK
PRRSFREE

图2A

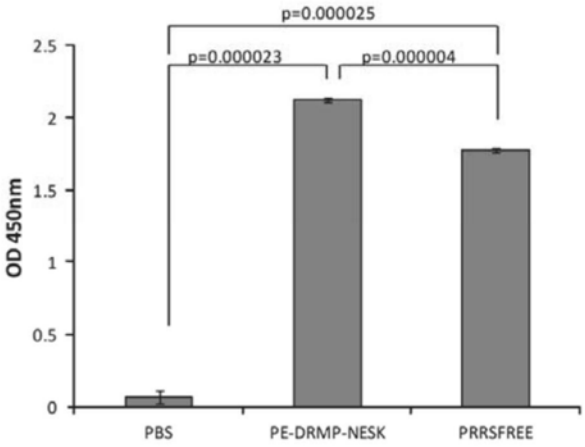

p=0.000025
p=0.000023
p=0.000004
OD 450nm
2.5
2
1.5
1
0.5
0
PBS
PE-DRMP-NESK
PRRSFREE

图2B

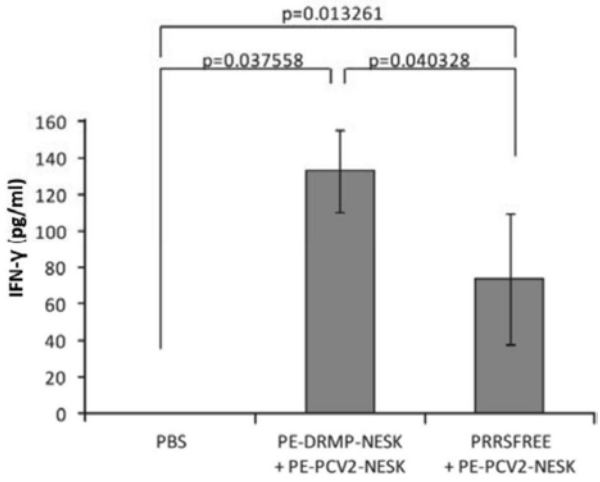

p=0.013261
p=0.037558
p=0.040328
IFN-γ (pg/ml)
160
140
120
100
80
60
40
20
0
PBS
PE-DRMP-NESK
+ PE-PCV2-NESK
PRRSFREE
+ PE-PCV2-NESK

图3A

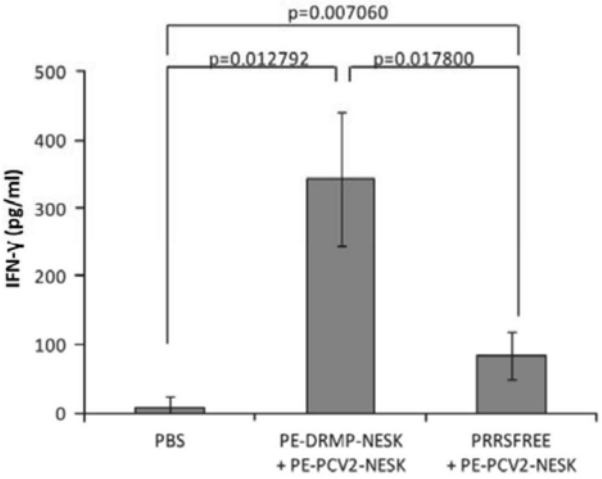

p=0.007060
p=0.012792
p=0.017800
IFN-γ (pg/ml)
500
400
300
200
100
0
PBS
PE-DRMP-NESK
+ PE-PCV2-NESK
PRRSFREE
+ PE-PCV2-NESK

图3B

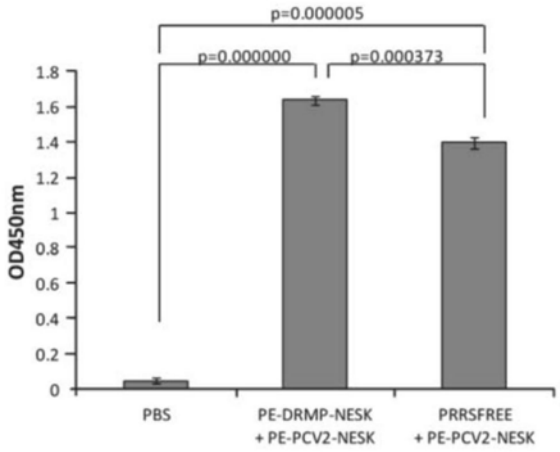

p=0.000005
p=0.000000
p=0.000373
OD450nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
PBS
PE-DRMP-NESK
+ PE-PCV2-NESK
PRRSFREE
+ PE-PCV2-NESK

图4A

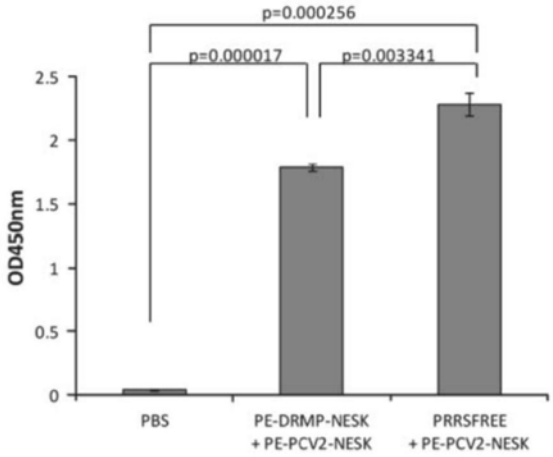

p=0.000256
p=0.000017
p=0.003341
OD450nm
2.5
2
1.5
1
0.5
0
PBS
PE-DRMP-NESK
+ PE-PCV2-NESK
PRRSFREE
+ PE-PCV2-NESK

图4B

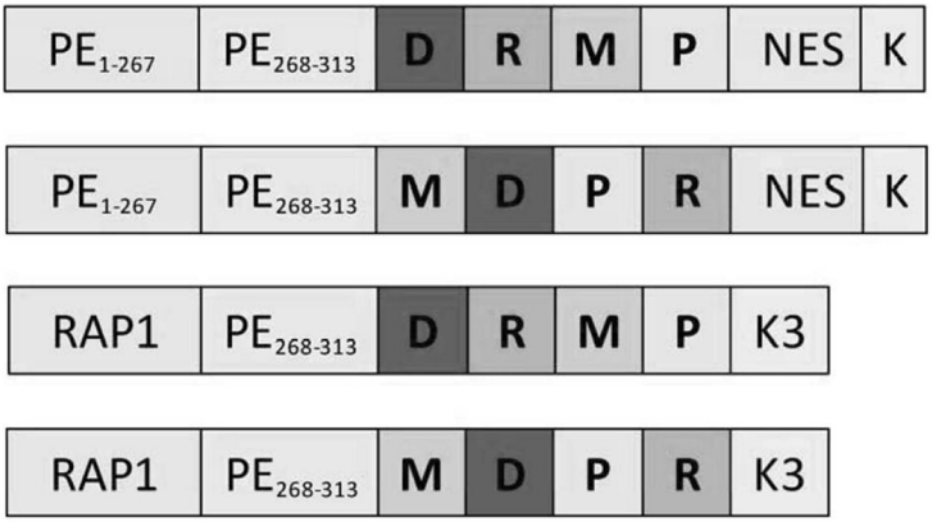

PE1-267
PE268-313
D
R
M
P
NES
K
PE1-267
PE268-313
M
D
P
R
NES
K
RAP1
PE268-313
D
R
M
P
K3
RAP1
PE268-313
M
D
P
R
K3

图6

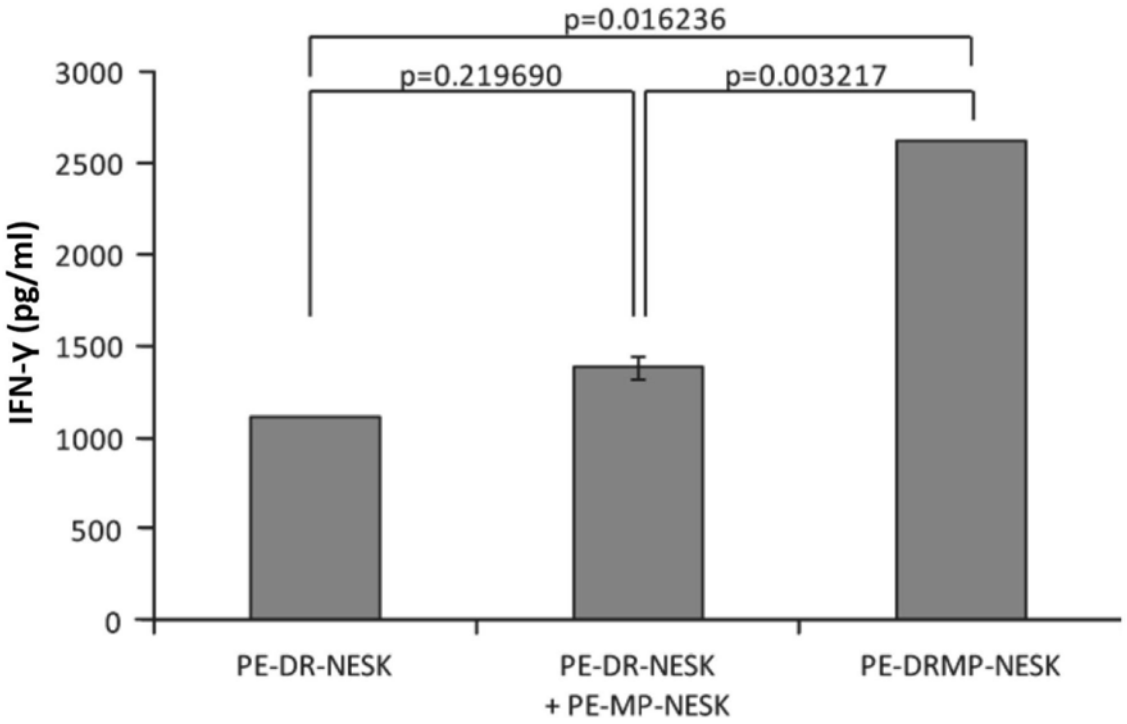

p=0.016236
p=0.219690
p=0.003217
3000
2500
2000
1500
1000
500
0
IFN-γ (pg/ml)
PE-DR-NESK
PE-DR-NESK
+ PE-MP-NESK
PE-DRMP-NESK

图5

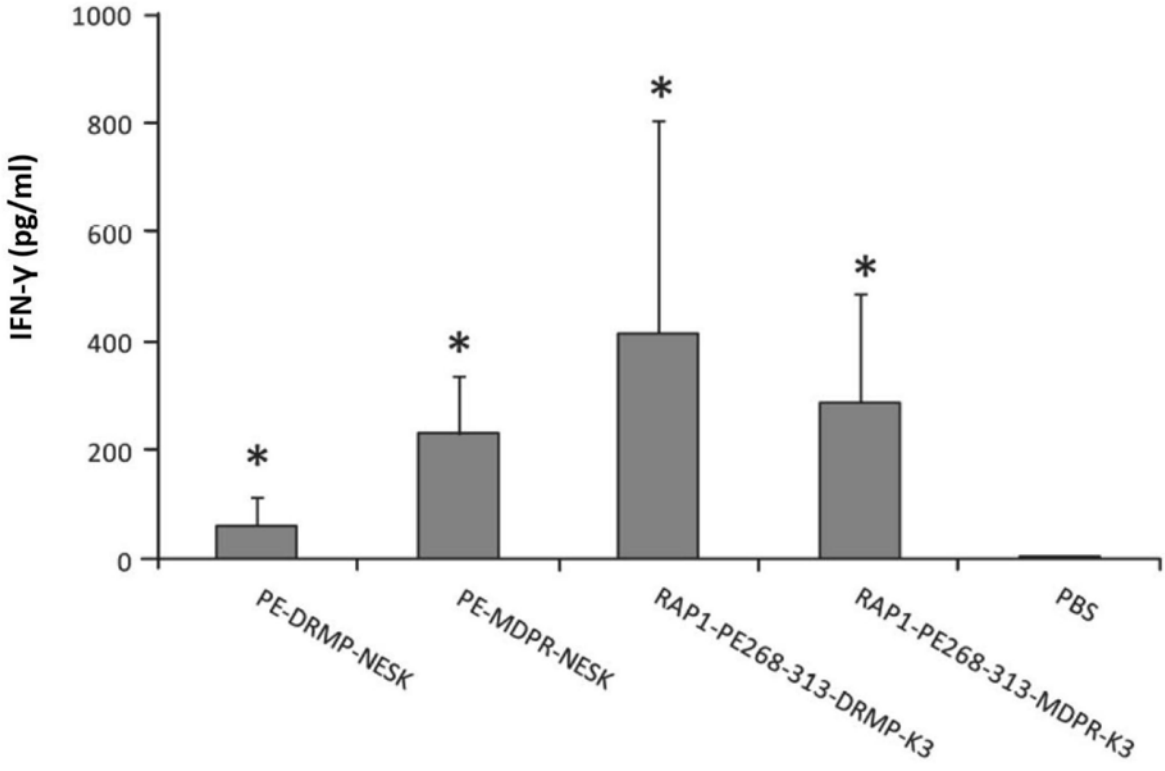

1000
800
600
400
200
0
IFN-γ (pg/ml)
*
*
*
*
PE-DRMP-NESK
PE-MDPR-NESK
RAP1-PE268-313-DRMP-K3
RAP1-PE268-313-MDPR-K3
PBS

图7